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Unexpected skin barrier influence from nonionic emulsifiers

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Abstract

Skin disorders are often treated with creams containing various active substances. The creams also contain emulsifiers, which are surface-active ingredients used to stabilize the emulsion. Emulsifiers are potential irritants and in the present study the influence of stearic acid, glyceryl stearate, PEG-2, -9, -40, and -100 stearate, steareth-2, -10 and -21 on normal as well as on irritated skin have been evaluated with non-invasive measurements. Test emulsions were created by incorporating 5% emulsifiers in a water/mineral oil mixture (50:50). The emulsions and their vehicle were then applied to normal skin for 48 h and to sodium lauryl sulfate (SLS) damaged skin for 17 h in aluminum chambers. Twenty-four hours after removal of the chambers the test sites were evaluated for degree of irritation. In normal skin, the emulsifiers induced significant differences in TEWL but not in skin blood flow. Five of the emulsifiers increased TEWL. In SLS-damaged skin an aggravation of the irritation was expected. However, no differences regarding skin blood flow was noted from the emulsifiers. Furthermore, three emulsifiers unexpectedly decreased TEWL. These results highlight the possibility of absorption of these emulsifiers into the lipid bilayer, which increase TEWL in normal skin and decrease TEWL in damaged skin.

Keywords: Surface-active agents; Nonionic emulsifiers; Normal skin; Surfactant-irritated skin; Barrier function.

1. Introduction

Topical preparations such as creams and ointments are widely used to treat various skin disorders. For instance, corticosteroids incorporated in creams are used for treating inflammatory dermatoses, such as hand eczema and atopic dermatitis. The preparations consist of an active drug in a vehicle, formulated mainly with consideration to cosmetic properties and stability. The vehicle is composed of water and emollients and is normally regarded as inactive. Emulsifiers are included in these creams, based on their ability to stabilize the product.

Ingredients for dermatological use are tested for skin irritation, according to OECD guidelines[1] for 4 h on intact skin on the back of albino rabbits.

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[1] OECD guidelines for the testing of chemicals. OECD, Publication Service, 2 Rue André Pascal, 75775 Paris Cedex 16, France.

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Studies are also often made on intact skin of volunteers, normally for 24–48 h (Cosmetic Ingredient Review, 1982, 1983, 1987, 1988; Walker et al., 1997). Assessment in human skin has the advantage that extrapolation from animal studies are not necessary. The grading of the skin reactions are usually done by visual examination of the test site, and the response scored on a categorical scale from 0 to 4 (Frosch and Kligman, 1979; Tupker et al., 1997). The response is generally a sporadic and rapidly declining erythema (Cosmetic Ingredient Review, 1982, 1983, 1987, 1988), of the same magnitude as from water. Also, biophysical instruments have been used in order to characterize the influence of different substances on the skin (van der Valk et al., 1984a,b; Agner et al., 1989; Kinnunen, 1992).

The aim of the present study was to investigate the structure–activity relationship of nine emulsifiers not only on normal but also on irritated skin. The skin reaction was assessed clinically and with non-invasive biophysical instruments. Two groups of chemically related nonionic emulsifiers were included in the test; stearic acid derivatives and stearyl alcohol derivatives with different ethylene oxide content, giving differences in hydrophilicity and molecular weight. Nonionic surfactants have long been recognized as those with the least potential for irritancy (Abraham, 1997). The term nonionic indicates that the molecule carries no charge at non-extreme pH levels. They were chosen since they are commonly used emulsifiers in medicinal and cosmetic formulations.

2. Materials and methods

2.1. Study population

The investigation comprised of two parts. In part I the emulsifiers were tested on normal skin of 20 volunteers (two men) and in part II on surfactant-irritated skin of 27 volunteers (seven men). Seventeen of the volunteers participated in both parts. The mean age was 38 years. The study was approved by the local ethics committee.

2.2. Chemicals

The substances used were nonionic surface-active agents, derived from stearic acid and stearyl alcohol. The following emulsifiers were included:

Stearic acid (SA) (Pristerine 9559 Unichema International, UK) hydrophile–lipophile balance (HLB) ca 3.

PEG-2 stearate (P2S) (Cithrol DEGMS N/E Croda Surfactant, UK) HLB 4.3

PEG-9 stearate (P9S) (Cremophor S9 BASF, Germany) HLB 12

PEG-40 stearate (P40S) (Myrj 52F ICI Surfactants, UK) HLB 16.9

PEG-100 stearate (P100S) (Crodet S100 Croda Chemicals, UK) HLB 18.8

Glyceryl stearate (GS) (Glyceroli Monostearas Ph Eur Danisco, Denmark) HLB 6.0

Steareth-2 (S2) (Volpo S2 veg Croda Chemicals, UK) HLB 4.9

Steareth-10 (S10) (Volpo S10 Croda Chemicals, UK) HLB 12.4

Steareth-21 (S21) (Cromul EM 1207 Croda Chemicals, UK) HLB 15.3

Polyethyleneglycol (PEG) stearates are ethoxylated carboxylic acids derived from stearic acid and ethylene oxide (EO). Glyceryl stearate is an ester of stearic acid and glycerine. Steareths are ethoxylated alcohols derived from stearyl alcohol and EO. The substances all have an equal carbon chain length of 18, and becomes more hydrophilic with increasing EO content, which is indicated by the number (i.e. PEG-9 stearate consists of an average of 9 mol EO) and the hydrophile–lipophile balance value (HLB). A higher HLB indicates a more hydrophilic compound. The materials were tested as emulsions in equal volumes water and mineral oil (LL Paraffin, Castrol Whitmor, UK), prepared by dissolving the substances with $HLB < 10$ in mineral oil and then adding water. The substances with $HLB > 10$ were dissolved in water, and then mineral oil was added. To create the emulsions, the solutions were heated on water bath and shaken using a Whirlmixer (Fisons Scientific Apparatus, Loughborough, Leicestershire, UK). The final concentration of the emulsifiers was 5% w/v. The control was composed of 50/50 v/v mineral oil/water.

2.3. Treatment

In part I, 50 μl of each emulsion was pipetted into large aluminium chambers (12 mm Finn Chambers, Epitest Oy, Finland) containing one layer of filter paper. The chambers were attached to the volar forearm skin with adhesive tape (Scanpore, Norgeplaster A/S, Oslo, Norway). After 48 h the patches were removed and the skin cleansed using a mild soap (ACO Mild Tvål, ACO HUD AB, Sweden). The application sites were randomized using Latin square.

In the second part of the study, skin irritation was induced before the emulsions were applied to the forearm. This was done by exposure to sodium lauryl sulfate (SLS, European Pharmacopoeia, 15% for 7 h) performed in the same way as described previously (Lodén and Andersson, 1996). Upon removal of the SLS-patches, the skin was gently rinsed with water and allowed to dry. The test substances were then randomly applied to the SLS-treated areas as above. After 17 h, the patches were removed and the skin cleansed as above.

2.4. Evaluation

Each site was examined 24 h after removal of the patch (day 2). Erythema was graded separately according to the following scale:

0, no reaction;
1, slight erythema, spotty or diffuse;
2, moderate erythema;
3, intense erythema.

TEWL was measured with an evaporimeter (Evaporimeter EP1, Servomed, Stockholm, Sweden) (Nilsson, 1977) equipped with a screen and grid to reduce air convection. The output was recorded by a computer, and the recording was made for 40 s after a stabilization period of 30 s. The microvascular blood flow was measured with a laser Doppler flowmeter (Periflux Laser Doppler Flowmeter, Perimed, Sweden) (Tenland 1982), and the results were registered on a printer (Servogor 120 BBC). The instrument was equipped with a special multifibre probe (PF 113 Integrating probe, Perimed) which has seven fibre triplets in the probe head, thus reducing variation due to spotty erythema. All measurements were carried out in a draught-free room at a temperature of 21–23°C and a relative humidity of 13–21%.

2.5. Statistics

The results are presented using box plots. The bottom line of the box is the first quartile (Q1), and the top is at the third quartile (Q3) value. A line is drawn across the box at the median. The whiskers are the line that extend from the top and bottom of the box to the lowest and highest observations that are still inside the region defined by the following limits:

Lower limit: Q1 – 1.5 (Q3–Q1)
Upper limit: Q3 + 1.5 (Q3–Q1)

Outliers are points outside of the lower and upper limits and are plotted with diamonds. To test the hypothesis that there were no differences in effect with respect to the variables TEWL, blood flow and visual erythema, among all substances within part I and part II, respectively, the non-parametric Friedman test was used. To test the hypothesis that there is no difference in the effect of the individual emulsifiers compared to control with respect to the two variables TEWL and skin blood flow, the non-parametric 1-sample Wilcoxon test median method was used. A fitted line regression analysis was performed to investigate the relationship between the mean TEWL and mean skin blood flow in normal and surfactant-irritated skin, respectively. A $P < 0.05$ was considered significant for all test methods. The software used was MINITAB® (Minitab, State College, PA).

3. Results

3.1. Clinical assessment

On normal and surfactant-irritated skin, slight erythema was found in some subjects (Table 1). There were no differences in erythema due to treatment with emulsifiers compared to control in any of the groups.

3.2. Instrumental evaluation

On normal skin, significant differences in effect of the different emulsifiers were found regarding TEWL ($P < 0.001$), but not in cutaneous blood flow ($P = 0.41$). Five of the applied substances significantly increased TEWL compared to the control (Fig. 1, Table 2).

Also when the substances were applied to SLS-irritated skin, significant differences in TEWL ($P < 0.001$) but not blood flow ($P = 0.074$) were found between treatments. However, TEWL was significantly lower after treatment with three of the emulsifiers compared to control while none increased TEWL (Fig. 2, Table 2).

3.3. Correlation between TEWL and blood flow

No correlation between the mean values of blood flow and TEWL was found on normal skin ($P = 0.32$). A significant correlation ($y = 4.43 + 0.243x$, $r^2 = 69.4\%$, $P = 0.002$) was found on surfactant-irritated skin.

Table 1
Scattered erythema were recorded after exposure to the emulsifiers

	Emulsifiers[a]									
	Control[b]	SA	GS	P2S	P9S	P40S	P100S	S2	S10	S21
Normal skin ($n = 20$)	0.5 (10)	0 (10)	0 (5)	1 (0)	1 (10)	1 (5)	0.5 (10)	1 (10)	1 (5)	1 (0)
Irritated skin ($n = 27$)	1 (15)	1 (41)	1 (22)	1 (41)	1 (26)	1 (41)	1 (22)	1 (30)	1 (44)	1 (19)

[a] SA, stearic acid; GS, glyceryl stearate; P2S, PEG-2 stearate; P9S, PEG-9 stearate; P40S, PEG-40 stearate; P100S, PEG-100 stearate; S2, steareth-2; S10, steareth-10; S21, steareth-21.

[b] Median values of clinical erythema. Within parenthesis, the number of observations with a score >1 is stated in percent.

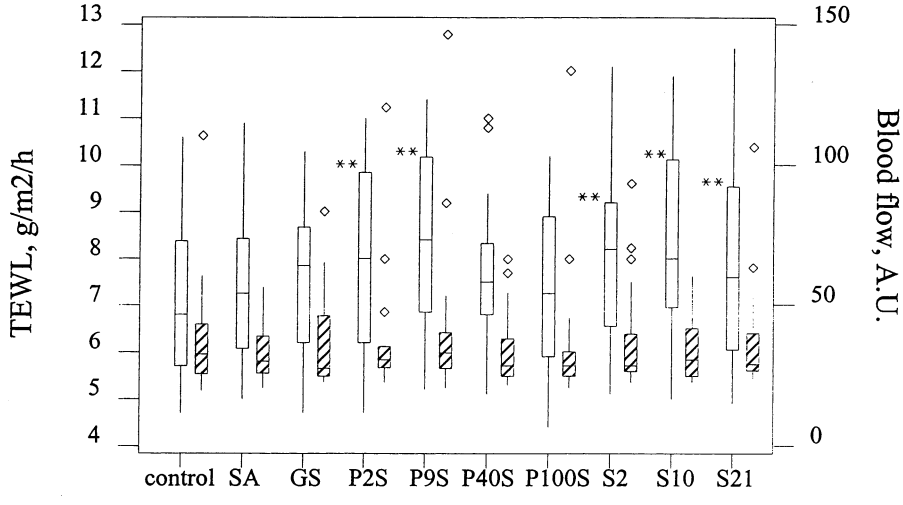

Fig. 1. The effect of the test substances on normal skin. Five emulsifiers increased TEWL compared with the control but skin blood flow was not different from control in any case. The boxplot shows TEWL (white boxes) and skin blood flow (striped boxes). The box is defined by the upper and lower quartiles and with the median marked by a subdivision of the box. The diamonds denotes outliers. ** $P < 0.01$. SA, stearic acid; GS, glyceryl stearate; P2S, PEG-2 stearate; P9S, PEG-9 stearate; P40S, PEG-40 stearate; P100S, PEG-100 stearate; S2, steareth-2; S10, steareth-10; S21, steareth-21.

Table 2
Three emulsifiers that increased TEWL in normal skin, decreased TEWL in irritated skin compared with the control[a]

Substance	*P* value TEWL normal skin[b]	*P* value TEWL SLS-irritated skin
Stearic acid	(0.173)	(0.110)
Glyceryl stearate	(0.162)	(0.486)
PEG-2 stearate	0.006↑	(0.949)
PEG-9 stearate	0.000↑	0.013↓
PEG-40 stearate	(0.059)	(0.809)
PEG-100 stearate	(0.794)	(0.313)
Steareth-2	0.002↑	0.008↓
Steareth-10	0.002↑	(0.151)
Steareth-21	0.006↑	0.000↓

[a] Statistical comparison of the differences between the effect of control and the effect of the emulsifiers on normal and SLS-irritated skin. The 1-sample Wilcoxon test median method was used. The areas were examined 24 h after removal of the test substances.

[b] Not significant *P* values within parenthesis. The arrows indicates increase (↑) or decrease (↓) in TEWL compared with the control.

4. Discussion

The skin is often exposed to surface-active agents like soaps, which may affect the skin barrier (van der Valk et al., 1984a,b; Tupker et al., 1989). Differences in the effects of surfactants have been investigated previously, e.g. using biophysical instruments (van der Valk et al., 1984a,b; Agner et al., 1989; Tupker et al., 1989; Goffin et al., 1995; Bárány et al., 1999). These investigations show that surfactants exert strong effects in experimental settings. SLS, a surfactant with a carbon chain length of 12, is ranked as the most irritating (Kligman and Wooding, 1967; Stillman et al., 1975; Wilhelm et al., 1994), and is often used as a model irritant (Tupker et al., 1997). Increasing the EO content of the molecule, resulting in a larger hydrophilic part, appears to decrease the irritation potential (Blake-Haskins et al., 1986; Rhein et al., 1986; Goffin et al., 1995; Bárány et al., 1999), possibly due to increased difficulties in penetration of the keratin matrix (Faucher and Goddard, 1978). Skin effects from emulsifiers have also been studied (Kinnunen, 1992), showing cetostearol and sorbitan sesquioleate applied in volatile solvents to significantly increase TEWL compared to control.

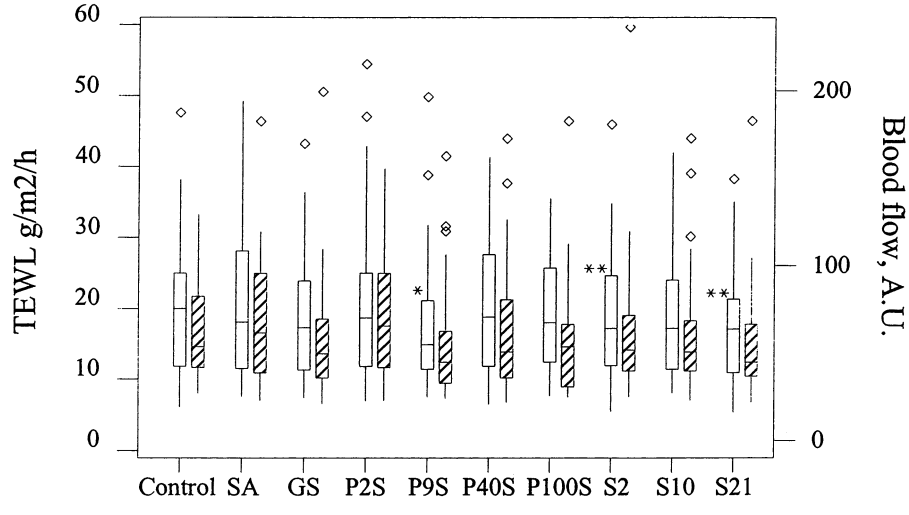

Fig. 2. The effect of the test substances on surfactant-irritated skin. Three emulsifiers decreased TEWL compared with the control but skin blood flow was not different from control in any case. The boxplot shows TEWL (white boxes) and skin blood flow (striped boxes). The box is defined by the upper and lower quartiles and with the median marked by a subdivision of the box. The diamonds denotes outliers. * $P < 0.05$, ** $P < 0.01$. SA, stearic acid; GS, glyceryl stearate; P2S, PEG-2 stearate; P9S, PEG-9 stearate; P40S, PEG-40 stearate; P100S, PEG-100 stearate; S2, steareth-2; S10, steareth-10; S21, steareth-21.

In the present study, the structure–effect relationship of some commonly used emulsifiers were evaluated using non-invasive bio-engineering methods. The skin was exposed to a single occlusive application of emulsions, consisting of the emulsifier in water and mineral oil. Commercial grade materials were used, which are mixtures of molecules of varying ethoxy and alkyl chain lengths distributed around a mean value. The mean alkyl chain length was an 18-carbon chain in all the investigated substances, and the hydrophilic group increased from 0 to 100 mol EO, thereby changing the molecules from lipophilic to hydrophilic. In the clinical evaluation of normal skin, only sporadic erythema were recorded, and no difference between the treatments were found. Thus, no overall structure–activity relation was noted (Cosmetic Ingredient Review, 1982, 1983, 1987, 1988). However, in the objective measurements, significant increases in TEWL were found for five emulsifiers with no corresponding increase in blood flow. An increased TEWL is a sensitive measure of barrier damage (van der Valk et al., 1984a,b; Agner and Serup, 1990) and an indication of the skin permeability (Lévêque 1989). Moreover, no correlation was found between the TEWL and blood flow values, indicating a skin barrier impairment without inflammatory response (Agner and Serup, 1989) possibly caused by direct alterations in the intercellular lipid membranes (Lévêque et al., 1993). These invisible changes did not manifest as redness or increased skin blood flow, but can increase the bioavailability of other substances due to fluidization or removal of lipid components of the SC (Ashton et al., 1986; Imokawa 1997). Increasing the fluidity of the highly structured lipid lamellae of the stratum corneum have been correlated with an increase in stratum corneum permeability, an effect that has been shown with some fatty acids with 18-carbon chains on transdermal salicylic acid flux (Golden et al., 1987).

In contrast to the findings in normal skin, a relationship was found between the TEWL and the superficial blood flow in surfactant-irritated skin. Unexpectedly, no further aggravation of the irritation was noted compared with the control, but instead TEWL was reduced significantly by three of the applied emulsifiers. A reduction in TEWL could indicate deposition of occlusive substances on the surface. However, the skin was cleansed with a soap, and the fact that the same three emulsifiers increased TEWL in normal skin argues against this assumption. Thus, it seems that these emulsifiers were able to penetrate into the skin and interact with its barrier function. It is also possible that the emulsifiers facilitated the absorption of mineral oil from the vehicle into the damaged skin. Previous studies show that petrolatum reduces TEWL in delipidized stratum corneum by forming a separate non-lamellar phase in the lipid bilayer (Ghadially et al., 1992). Other more hydrophilic lipids also seems able to reduce SLS-irritation (Lodén and Andersson, 1996) and to promote barrier recovery (Mao-Qiang et al., 1996).

In conclusion, the present study confirms that commonly used emulsifiers induce sporadic erythema in exposed normal skin. However, independent of the erythema, increased TEWL was induced by some of the emulsifiers, indicating an invisible impairment of the stratum corneum barrier function. Possible consequences of this are enhanced absorption of applied drugs, but also of substances toxic to the skin. Interestingly, the emulsifiers that affected SLS-damaged skin were the same as those affecting normal skin, but noteworthy here reduced the experimentally increased TEWL. Thus, one might speculate about the possible use of emulsifiers as a regulator of the bioavailability of drugs, i.e. to increase the absorption in skin with low permeability and to decrease the absorption in skin with high permeability.

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