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O/W Emulsions compromise the stratum corneum barrier and improve drug penetration

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Background: W/O emulsions improve the stratum corneum barrier, while microemulsions tend to compromise it. We, therefore, were interested to explore the effects of O/W emulsions on the stratum corneum barrier. *Methods:* Aqueous Cream BP 2001, Clioquinol Cream BP 1999 without clioquinol, Nonionic Hydrophilic Cream DAB 2001 without glycerol, Hydrophilic Skin Emulsion Base NRF S. 25., point of time 2001, without glycerol, and Base Cream DAC were tested versus untreated controls in 29 healthy volunteers for 7 days. Outcome measures included transepidermal water loss (TEWL), skin redness (chromametry a*-value) and erythrocyte circulation in the subpapillary vessels (laser Doppler). Barrier compromise was subsequently explored by performing the hydrocortisone blanching test using Hydrocortisone Cream 0.5% NRF 11.36. (outcome measure: a*-value) in 15 subjects and the sodium lauryl sulfate (SLS) irritation test (outcome measures: TEWL, a*-value, laser Doppler) in 14 subjects. *Results*: Pretreatment with the test emulsions produced increases in TEWL (statistically significant for all test emulsions), a*-value (statistically significant for Aqueous Cream BP 2001 and Base Cream DAC), and laser Doppler value (statistically significant for all emulsions except Base Cream DAC). Hydrocortisone penetration was statistically significantly increased with all test emulsions versus untreated contols. SLS irritation was mostly statistically significantly increased versus untreated controls when analyzing the study endpoint-baseline difference. *Conclusions:* O/W emulsions may compromise the stratum corneum barrier and improve drug penetration.

1. Introduction

Emulsifiers in aqueous solution produce dehydration and compromise the stratum corneum barrier [1, 2]. However, the underlying mechanism is not easy to understand. Predominantly hydrophilic emulsifiers would clearly appear to cause partial loss of stratum corneum lipids, whereas this appears quite unlikely with predominantly lipophilic emulsifiers such as those used in W/O emulsions [3]. Fartasch [4] compared toluene with 0.5% and 1% sodium lauryl sulfate. Toluene removed most of the epidermal lipids, damaging their lamellar structure. Sodium lauryl sulfate caused no barrier lipid damage detectable by electron microscopy. In fact, the lamellar structures were readily detectable. Similar results were obtained by Froebe et al. [5] in *in vitro* experiments with 0.1% to 2% sodium lauryl sulfate. They found that no ceramides and only 8% or 15% of free fatty acids or cholesterol were removed by 2% sodium lauryl sulfate. Identical findings were obtained by Lévêque et al. [6] in *in vitro* experiments using 1% sodium lauryl sulfate. Again, there was no reduction in polar lipids and only a slight reduction in nonpolar lipids, whose contribution to the stratum corneum barrier is small.

Of great relevance are studies by Downing et al. [7] showing that sodium lauryl sulfate can be incorporated into liposomes from stratum corneum lipids up to a concentration of 18%. Moreover, the liposomes proved to be very stable even with a sodium lauryl sulfate concentration of 20%. The liposomes with a high sodium lauryl sulfate content proved to be substantially more penetrable to glucose than liposomes without sodium lauryl sulfate. Very similar findings were obtained by de la Maza et al. [8] and López et al. [9]. However, instead of glucose penetration, they measured carbofluorescein release from liposomes similar in composition to stratum corneum lipids. Emulsifiers studied included sodium lauryl sulfate, anionactive sodium dodecyl ether sulfate, nonionic octylphenol ethoxylated with 10 units of ethylene oxide (Triton X 100), and amphoteric dodecyl betaine. Quantitative differences were found between the emulsifiers, but results were essentially identical to the findings of Downing et al. [7]. It is therefore quite conceivable that O/W emulsifiers should be incorporated into stratum corneum lipid lamellae *in vivo*, thus significantly improving the permeability of the latter. Also conceivable is the incorporation of W/O emulsifiers into epidermal lamellar lipid structures. However, as these have similar properties to the physiologic barrier lipids, W/O emulsifier incorporation would be rather unlikely to produce a significant change in lipid lamellar permeability.

However, there is yet another mechanism by which emulsifiers can impact on stratum corneum function. Following exposure to sodium lauryl sulfate, Fartasch [4] found a

monolayer transit cell zone as evidence of immature cell differentiation and lipid droplets in the corneocyte matrix. Parakeratosis may occur as well. The author hypothesizes that there may be disruption of the lamellar body-synthesizing system. Sodium lauryl sulfate would thus tend to interfere with the renewal of lamellar lipid structures rather than with existing lipid structures. Disruption of keratinocyte differentiation is also thought to be involved. It is conceivable for this process to interfere with fillagrin synthesis, possibly reducing the content of water-binding amino acids in the stratum corneum. This may explain dehydration of the stratum corneum following surfactant exposure. Earlier studies by other authors point in the same direction [10, 11].

Moreover, sodium lauryl sulfate and presumably other surfactants bind directly to corneocyte keratins, causing denaturation of the latter [4]. This effect may give rise to swelling of stratum corneum cells [12]. The direct effect of surfactants on stratum corneum keratins also manifests as a "keratolytic" effect. Thus, Weiss et al. [13] and Gloor and Beier [14] observed thinning of the stratum corneum after surfactant exposure. In a recent publication, Gloor et al [15] report a "keratolytic" effect of a hydrophilic and a lipophilic microemulsion. Similarly, the corneosurfometry findings reported by Gabard et al. [16] and Piérard et al. [17] would appear to be due to a direct impact of surfactants on corneocytes. The characteristic appearance of surfactant-damaged skin is likely to be due, at least in part, to this effect.

While the emulsifiers in an aqueous base undoubtedly have dehydrating and barrier-compromising effects [1, 2], this is not so clearly the case for emulsion vehicles. Schäfer and Redelmeier [1] hypothesized that micelle formation in the base of a topical dermatological product reduces the stratum corneum-compromising potential of emulsifiers.

W/O emulsions appear not to compromise the horny layer. In a short-term study, Bettinger et al. [18] performed a standardized washing procedure with sodium lauryl sulfate solution following pretreatment with a W/O emulsion and found that stratum corneum water content was increased at the start of washing and, unlike an untreated comparator site, did not fall below baseline in response to washing. In a long-term (6-week) study, Gloor and Gehring [19] found that treatment with Hydrous Lanolin Alcohol Ointment DAB increased stratum corneum water content and reduced transepidermal water loss (TEWL), a measure of the barrier function of the horny layer. This suggests that W/O emulsions afford preventive skin protection via an occlusive effect and, even when used for prolonged periods of time, will not compromise the stratum corneum barrier.

The effects of O/W emulsions are unclear. Bettinger et al. [20] found a moisturizing or a lacking effect for 5 O/W emulsions of very different compositions. Following a standardized washing procedure with sodium lauryl sulfate (SLS) after pretreatment with the O/W emulsions, the dehydration effect of the wash solution following pretreatment with the O/W emulsions was reduced rather than increased versus untreated controls. A long-term study using Nonionic Hydrophilic Cream DAB without glycerol may have produced evidence of barrier compromise, but this evidence is flawed by failure to include an untreated control [19]. A lipophilic microemulsion and a hydrophilic microemulsion, used for 4 days, have both been shown to produce dehydration of the horny layer and an increase in TEWL [21].

The present study used 5 pharmacopoeial formulations to determine whether O/W emulsions may damage the stratum corneum. Outcome measures included the effects of treatment on TEWL, skin redness (chromametry a*-value), and erythrocyte circulation in the subpapillary vessels (laser Doppler). In addition, some subjects underwent the hydrocortisone blanching test after one week's pretreatment (a*-value). Finally, the SLS irritation test was performed in some subjects after one week's treatment, determining the impact of irritation on TEWL, skin redness (a*-value), and erythrocyte circulation in the subpapillary vessels (laser Doppler).

2. Investigations and results

2.1. Changes in TEWL and subpapillary blood flow during 7 days' treatment with 5 different O/W emulsions

Twenty-nine healthy volunteers had the following study products applied to 5 test areas on one arm twice daily:

- Aqueous Cream BP 2001
- Clioquinol Cream BP 1999 without clioquinol
- Nonionic Hydrophilic Cream DAB without glycerol
- Hydrophilic Skin Emulsion Base NRF S. 25. without glycerol
- Base Cream DAC

The contralateral area was left untreated. Treated/untreated sides were reversed from subject to subject. TEWL and skin redness (chromametry a*-value) were measured before the start and at the end of treatment. Fourteen subjects also underwent a laser Doppler study. Study endpoint-baseline differences were analyzed.

All study O/W emulsions produced a significant increase in TEWL. Two test emulsions gave rise to a statistically significant increase in the a*-value, which also tended to increase with the other three study emulsions. All test emulsions except Base Cream DAC produced significant increases in laser Doppler readings versus untreated controls (Figs. 1 and 2). These data suggest that the study emulsions compromised the skin barrier and produced irritative hyperemia in the subpapillary vessels.

2.2. Hydrocortisone blanching test after 7 days' treatment with the 5 O/W emulsions

Fifteen subjects then underwent the hydrocortisone blanching test using Hydrocortisone Cream 0.5% NRF 11.38 and 24 h occlusion. The changes from pre-blanching test (day 7) to post-blanching test (day 8) were analyzed as well as the changes from study baseline (Day 0) to post-blanching test (day 8).

The a*-value showed a blanching-induced decrease on all test sites. The observed reductions were statistically significantly greater on the O/W emulsion-treated sites than on the untreated sites for all test emulsions for the day 8-day 7 difference and for all emulsions except Base Cream DAC for the day 8-day 0 difference (Fig. 3). This observation suggests that O/W emulsion pretreatment increased hydrocortisone penetration.

2.3. SLS Irritation test after 7 days' treatment with the 5 O/W emulsions

After pretreatment with the 5 O/W emulsions, 14 subjects underwent the SLS irritation test. TEWL, a*-value, and laser Doppler readings were obtained before and after this

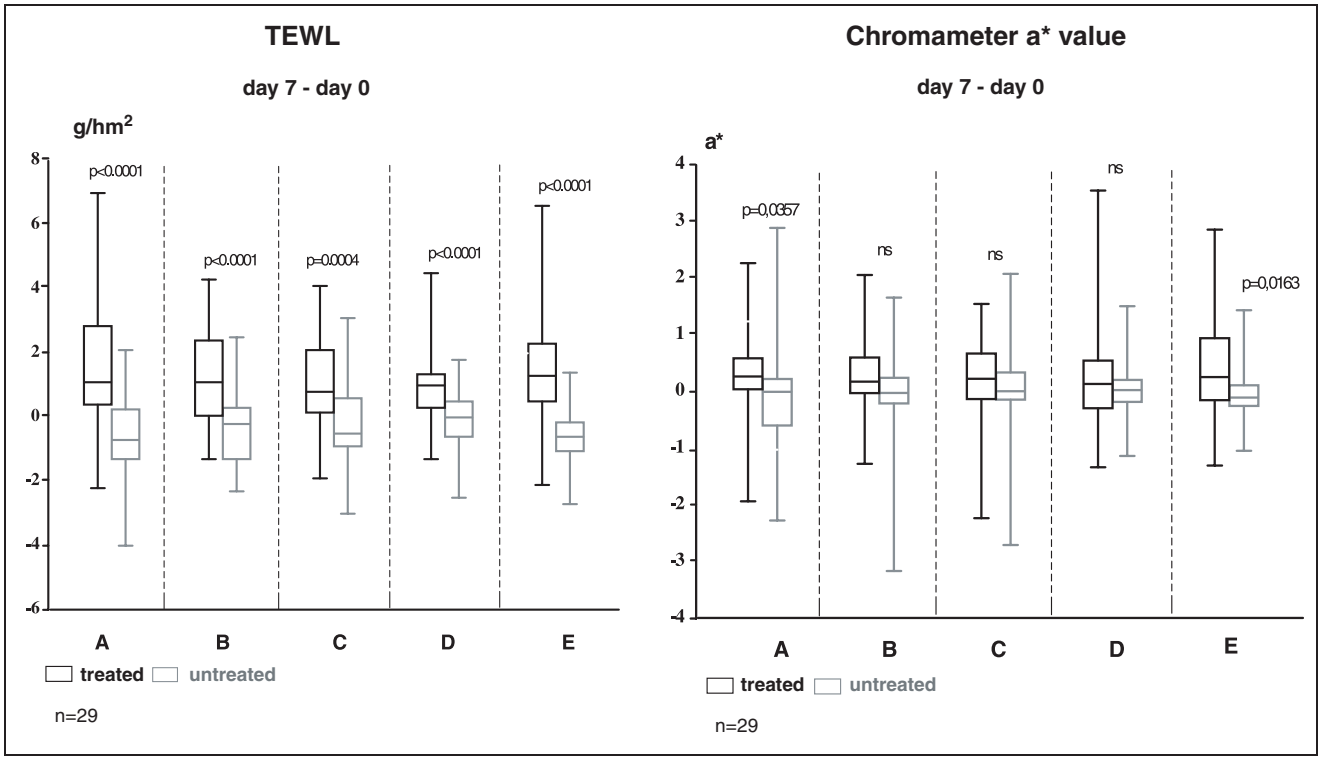

Fig. 1: Impact of 7 days' treatment with 5 different O/W emulsions on TEWL and a*-value versus untreated. Comparison of day 7-day 0 differences on the treated and untreated sites Data are presented as medians, boxes (25% and 75% percentiles), minima, and maxima. The test areas treated with the 5 test emulsions showed an increase in TEWL as evidence of barrier compromise and an increase in a*-value as evidence of irritative hyperemia. N = 29, A: Aqueous Cream BP 2001, B: Clioquinol Cream BP 1999 without clioquinol, C: Nonionic Hydrophilic Cream DAB 2001 without glycerol, D: Hydrophilic Skin Emulsion Base NRF S. 25. without glycerol, E: Base Cream DAC

test. The changes from pre-SLS irritation test (day 7) to post-SLS irritation test (day 8) were analyzed as well as the changes from study baseline (day 0) to post-SLS irritation test (day 8).

TEWL, a*-value, and laser Doppler readings mostly showed statistically significant increases versus untreated comparator sites for the day 8-day 0 comparison, which reflects the situation encountered in "real life" where barrier compromise from O/W emulsion use and barrier compromise from washing with surfactant solutions are additive. The SLS irritation test *per se* (day 8-day 7 comparison) consistently showed further increases in TEWL, a*-value, and laser Doppler readings. The observed increases were statistically significant on all outcome measures for Base Cream DAC and on the TEWL for Hydrophilic Skin Emulsion Base NRF S. 25. (without glycerol) (Figs. 4–6).

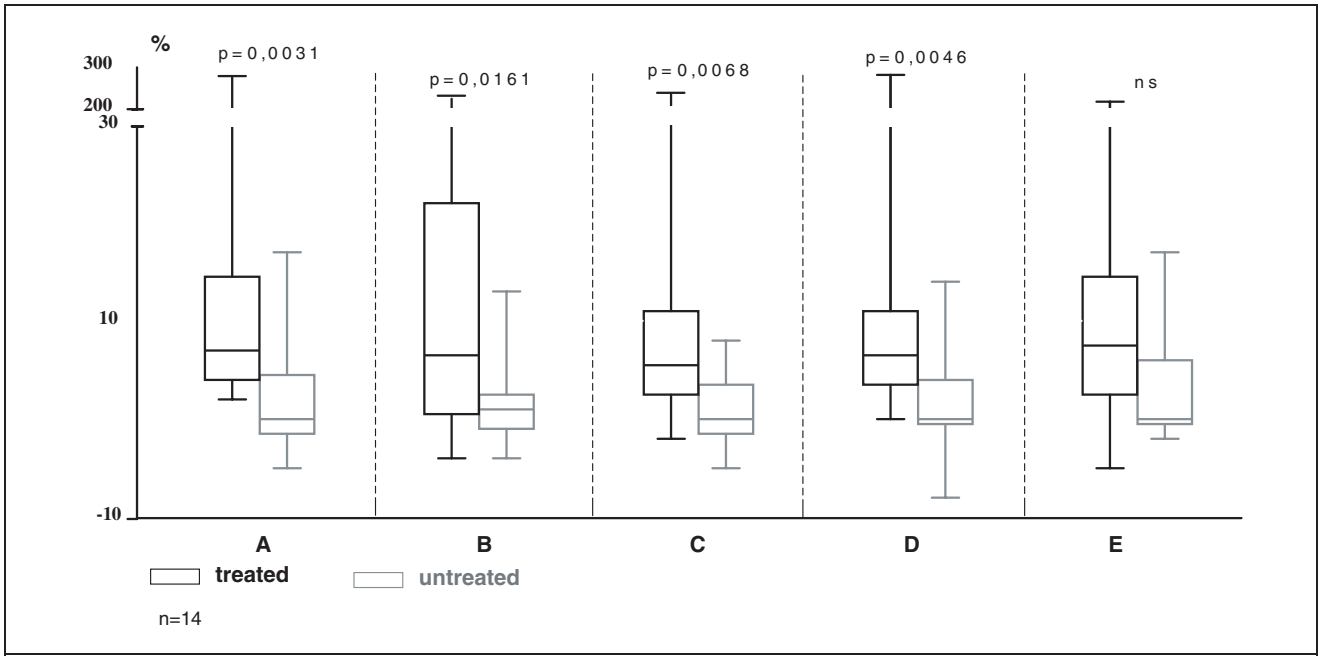

Fig. 2: Impact of 7 days' treatment with 5 different O/W emulsions on laser Doppler readings versus untreated. Comparison of day 7-day 0 differences on the treated and untreated sites. Data are presented as medians, boxes (25% and 75% percentiles), minima, and maxima. The test areas treated with the 5 test emulsions showed an increase in laser Doppler readings as evidence of irritative hyperemia. N = 14. For key to symbols see Fig. 1

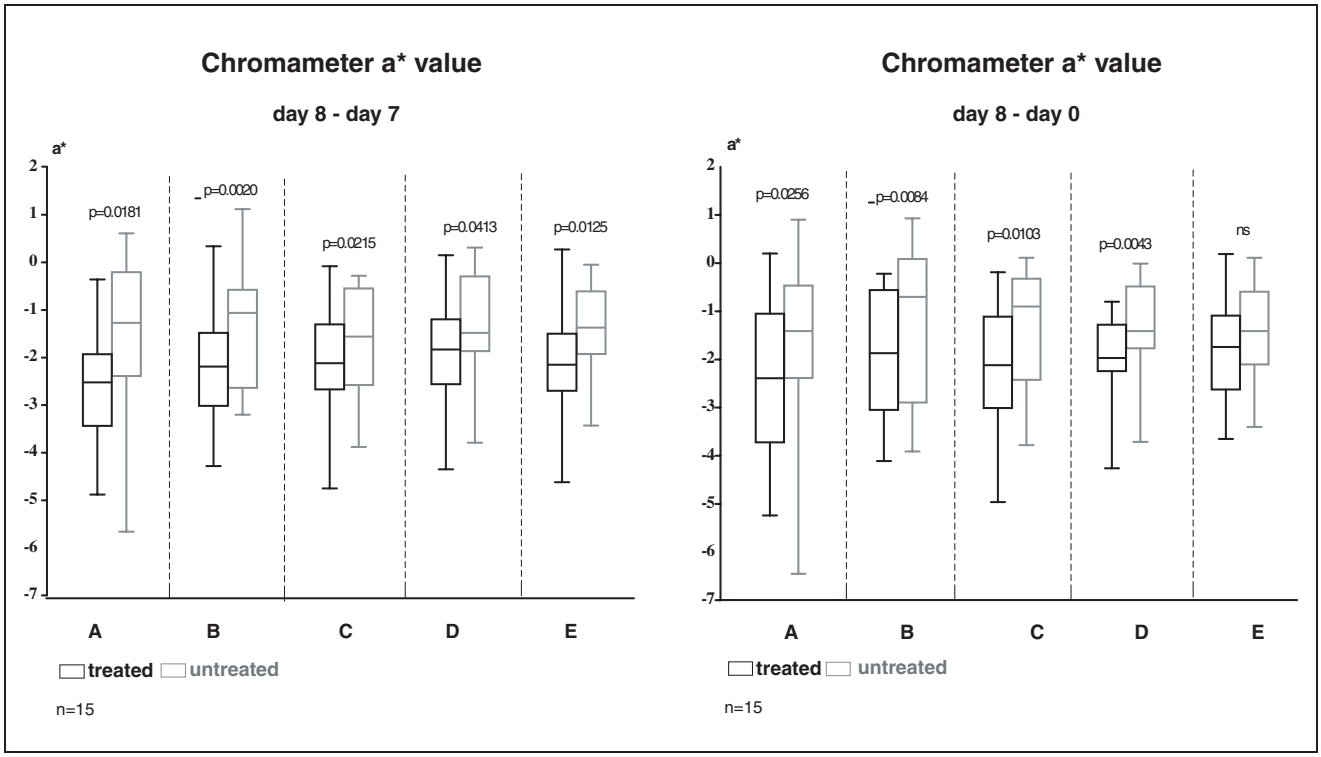

Fig. 3: Hydrocortisone blanching test: Change in a*-value on the sites pretreated with 5 different O/W emulsions versus untreated. Comparison of day 8-day 7 and day 8-day 0 differences on the treated and untreated sites. Data are presented as medians, boxes (25% and 75% percentiles), minima, and maxima. While both the treated and the untreated sites showed blanching, this effect was substantially greater on the emulsion-treated sites N = 15. For key to symbols see Fig. 1

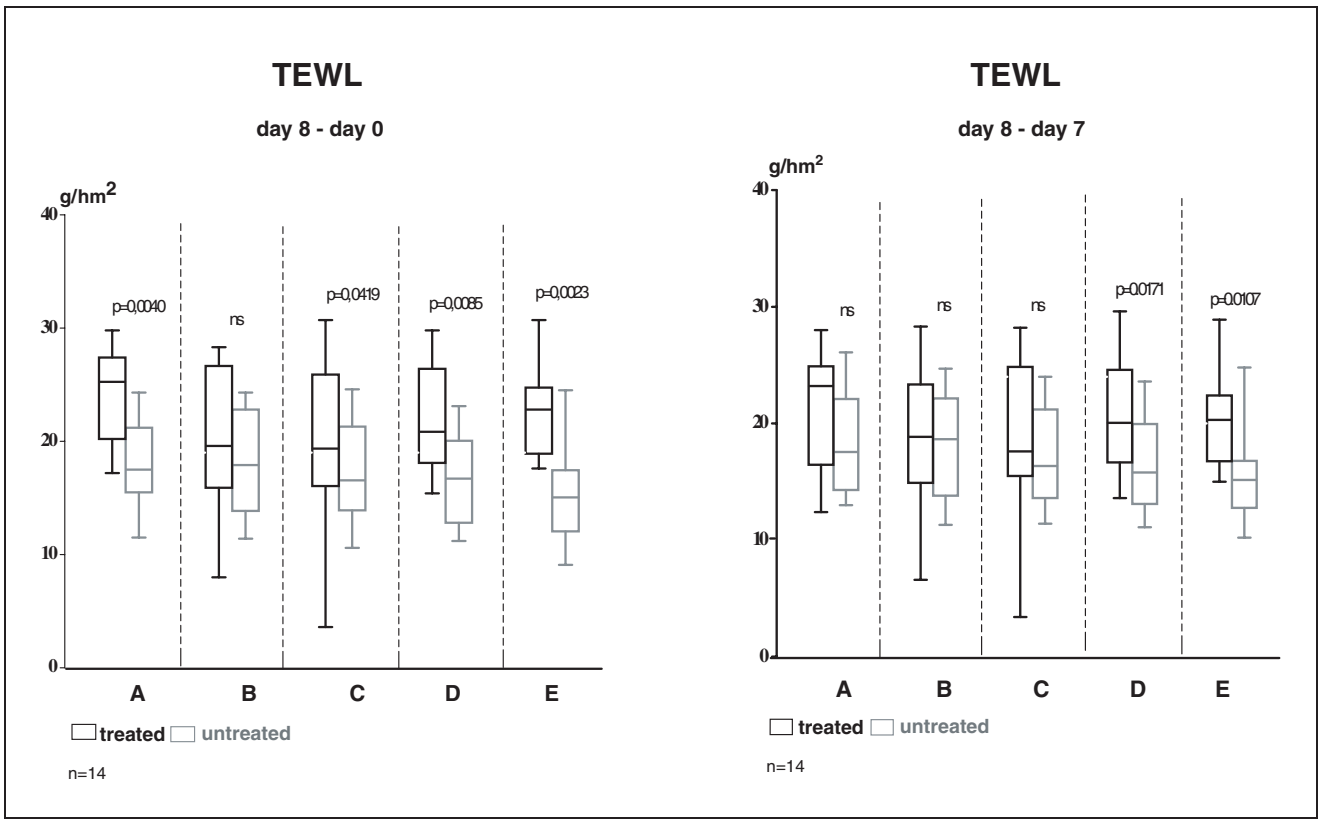

Fig. 4: Sodium lauryl sulfate (SLS) irritation test: impact on TEWL. Comparison of day 8-day 7 and day 8-day 0 differences on the treated and untreated sites. Data are presented as medians, boxes (25% and 75% percentiles), minima, and maxima. While both the treated and the untreated sites showed increases in TEWL, this effect was more pronounced on the treated sites. Some differences were statistically significant. N = 14. For key to symbols see Fig. 1

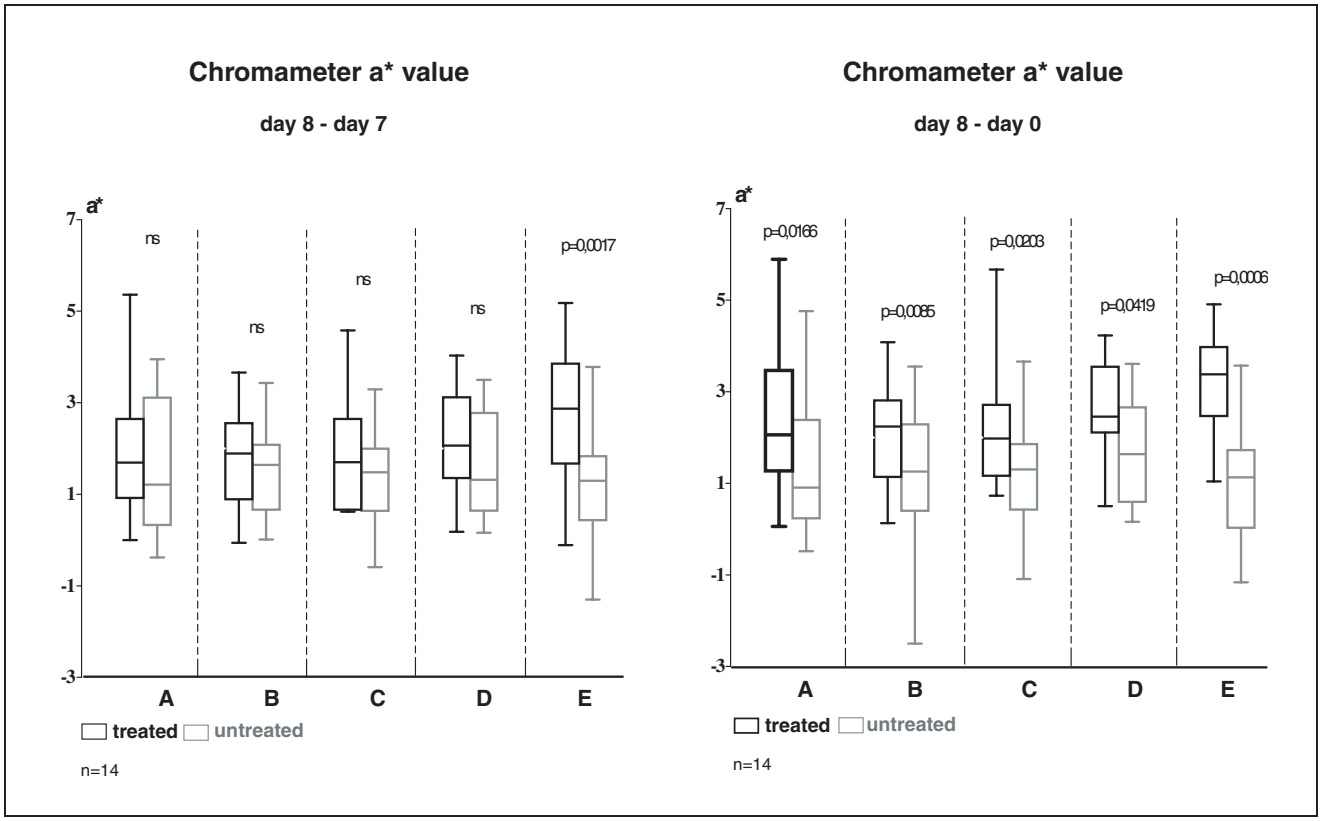

Fig. 5: Sodium lauryl sulfate (SLS) irritation test: impact on a*-value. Comparison of day 8-day 7 and day 8-day 0 differences on the treated and untreated sites. Data are presented as medians, boxes (25% and 75% percentiles), minima, and maxima. While both the treated and the untreated sites showed increases in the a*-value, this effect was more pronounced on the treated sites. Some differences were statistically significant. N = 14. For key to symbols see Fig. 1

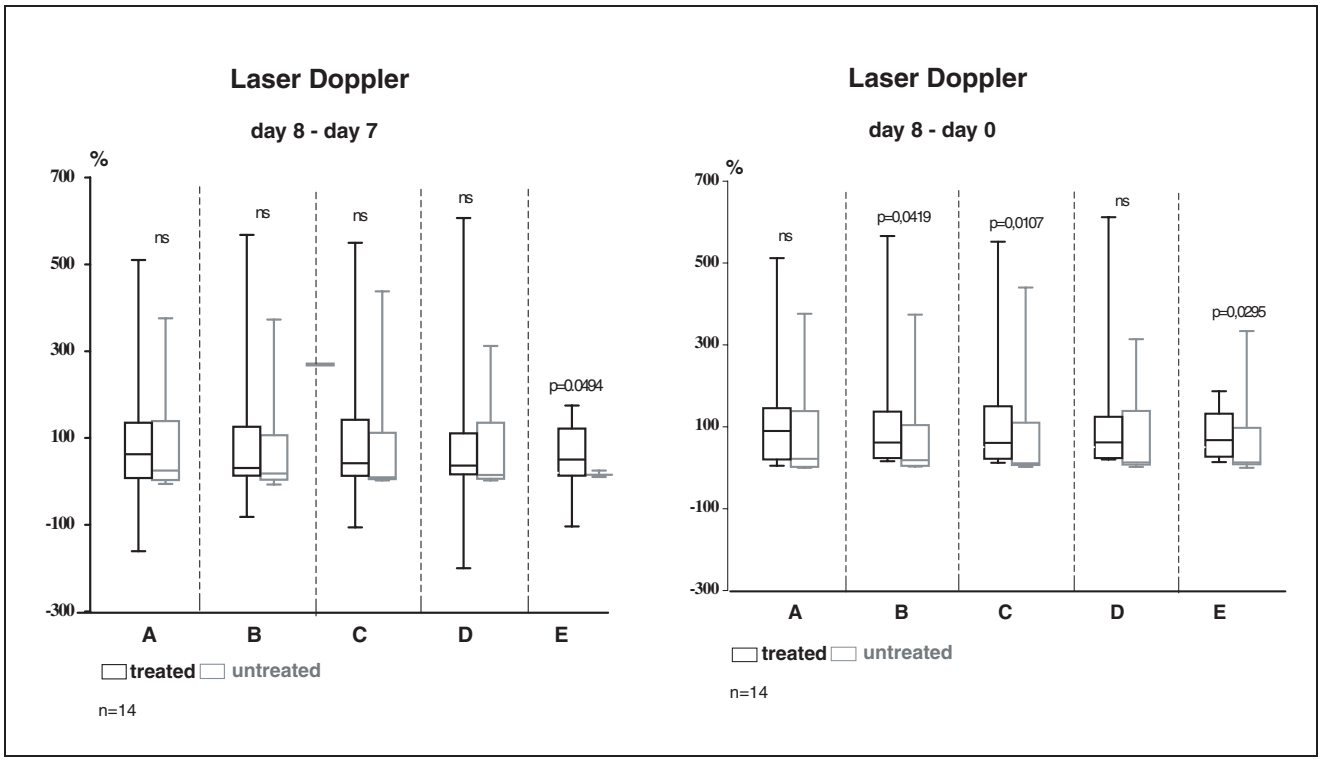

Fig. 6: Sodium lauryl sulfate irritation (SLS) test: impact on laser Doppler readings. Comparison of day 8-day 7 and day 8-day 0 differences on the treated and untreated sites. Data are presented as medians, boxes (25% and 75% percentiles), minima, and maxima. While both the treated and the untreated sites showed increases in laser Doppler readings, this effect was more pronounced on the treated sites. Some differences were statistically significant. N = 14. For key to symbols see Fig. 1

3. Discussion

O/W Emulsions are the most popular vehicles for topical dermatologicals because of their excellent penetrability, resulting in the superior acceptance of these products. Moreover, O/W emulsions improve drug penetration, as confirmed in the hydrocortisone blanching test in the present study. Like all topical dermatological corticosteroids, hydrocortisone produces skin blanching. The extent of the blanching correlates with the amount of penetrated drug. At low drug penetration volumes, the hydrocortisone blanching test can therefore be used to estimate the permeability of the stratum corneum barrier to topical dermatological corticosteroids [22]. Our blanching test results are consistent with the observed increase in TEWL after 2 weeks' treatment with the study O/W emulsions, since TEWL, measuring the amount of water lost through the stratum corneum, is also a measure of the barrier function of the horny layer. A methodological problem was that, due to pretreatment with the O/W emulsions, the treated test areas showed higher preblanching test a*-values than did the untreated comparator sites. To estimate the potential bias that might have resulted from this difference in blanching test baseline conditions, we analyzed the day 8-day 0 difference in addition to the day 8-day 7 difference in a*-value readings. However, the day 8-day 0 difference also showed statistically significantly greater a*-value reductions on the O/W emulsion-treated sites for all test emulsions except Base Cream DAC. We, therefore, can rule out methodology-related errors.

Another important consideration is stratum corneum barrier compromise in patients with atopic dermatitis, a skin condition characterized by changes in horny layer lipids in the intercellular spaces. There is a lack of ceramides, ceramide 1 and cermide 2 in particular [23, 24]. Ceramide 1 is thought to have a key role in maintaining stratum corneum barrier function. TEWL is therefore increased in atopic dermatitis sufferers [25–27]. The effects of O/W emulsions demonstrated in the present study may have adverse effects in patients with atopic dermatitis. In fact, all five study O/W emulsions produced a statistically significant increase in TEWL after 7 days' treatment with these emulsions, thus strongly suggesting deterioration of stratum corneum barrier function. Moreover, the observed increase in the a*-value – although it was statistically significant only for Aqueous Cream BP and Base Cream DAC-along with the observed increase in laser Doppler readings, which was statistically significant for all test emulsions except Base Cream DAC, shows that O/W emulsions may also produce irritative hyperemia in the subpapillary vessels.

The SLS irritation test revealed a statistically significantly greater increase in TEWL for Hydrophilic Skin Emulsion Base NRF S. 25. without glycerol and Base Cream DAC and statistically significantly greater increases in a*-value and laser Doppler readings for Base Cream DAC versus untreated controls. However, as pretreatment-induced irritation had caused different SLS irritation test baseline values between treated and untreated test areas, we additionally analyzed the day 8 (study endpoint)-day 0 (study baseline) difference in SLS irritation test results. This analysis also reflects the situation encountered in "real life" where barrier compromise from cream use and barrier compromise from washing with surfactant solutions are additive. This analysis revealed statistically significant increases in TEWL for all test emulsions except Clioquinol Cream BP 1999 without clioquinol, statistically significant increases in the a*-value for all test emulsions, and statistically significant increases in laser Doppler readings for all test emulsions except Aqueous Cream BP 2001 and Hydrophilic Skin Emulsion Base NRF S.25. without glycerol. Barrier compromise and irritation are therefore very likely to have occurred. Prolonged use of O/W emulsions thus appears highly problematic in patients with atopic dermatitis and where drug-free formulations are used.

The situation is altogether different once glycerol or urea is added to an O/W emulsion. In healthy volunteers subjected to repeated washings over 8 days, Grunewald et al. [28] found that intermittent treatment with an O/W emulsion containing glycerol or urea substantially reduced the adverse effects of washing. Studies by Bettinger et al. [29] and Fluhr et al. [30] have demonstrated that glycerol confers regenerative skin protection. Gloor and Gehring [19] showed that improvement in horny layer barrier function, demonstrated by TEWL reduction, was also maintained after 6 weeks' treatment with an O/W emulsion containing glycerol. Therefore, the above observations apply only to glycerol-free O/W emulsions (in the German Pharmacopoeias Hydrous Hydrophilic Ointment DAB, Base Cream DAC; in the British Pharmacopoeia Aqueous Cream BP 2001; in the US-Pharmacopoeia Hydrophilic Ointment USP25/NF20). However, most O/W emulsions in German Pharmacopoeias contain glycerol, and these, as a function of glycerol content, produce only a low or no barrier compromise or a barrier improvement. On the other hand, they may have the drawback of lower drug penetration.

Interestingly, 5 study O/W emulsions, while differing greatly in composition, produced no dehydration [20]. This would appear to be due to the availability of an ample moisture supply from the free water of the O/W emulsion, thus masking the impact of epidermal lipid compromise on stratum corneum moisture content.

4. Experimental

4.1. Study population

Twenty-nine healthy volunteers 18 years of age and older were enrolled in the study. Exclusion criteria were pregnancy, lactation, medical conditions, use of chronic medications, skin conditions, and use of any skin cleansing or skin care products or topical dermatologicals 4 days before and during the study. The protocol was reviewed and approved by the Independent Ethics Committee of the University of Freiburg, Freiburg, Germany, based on the guidelines of the Declaration of Helsinki.

4.2. Study emulsions

Aqueous Cream BP 2001: Purified water 69.9, cetylstearyl alcohol 8.1, phenoxyethanol 1.0, sodium lauryl sulfate 0.9, liquid paraffin 6.0, white soft paraffin 15.0. This anionic formulation is similar to Hydrous Hydrophilic Cream DAB, but the BP formulation contains sodium lauryl sulfate (contained in emulsifying cetylstearyl alcohol type B) instead of sodium cetyl stearyl sulfate (contained in emulsifying cetylstearyl alcohol type A).

Clioquinol Cream BP 1999 without clioquinol: White soft paraffin 15.0, cetylstearyl alcohol 7.2, macrogol-22-cetostearyl ether 1.8, liquid paraffin 6.0, chlorocresol 0.1 purified water ad 100.0. This formulation is similar to Cetomacrogol Lotion FNA.

Nonionic Hydrophilic Cream DAB 2001: without glycerol (replaced with water): polysorbate 20 5.0, cetylstearyl alcohol 10.0, white soft paraffin 25.0, purified water 60.0.

Hydrophilic Skin Emulsion Base NRF S. 25. (point of time 2001): without glycerol (replaced with water): Sorbitan monostearate 2.0, macrogol-9-stearate 3.0, medium chain triglycerides 5.0, anhydrous citric acid 0.07, potassium sorbate 0.14, purified water ad 100.0.

Base Cream DAC: Glycerol monostearate 60 4.0, cetyl alcohol 6.0, medium chain triglycerides 7.5, white soft paraffin 25.5, macrogol-20-glycerol monostearate 7.0 propylene glycol 10.0, purified water 40.0. Propylene glykol was not removed since evidence of its effect on the barrier function of the horny layer is lacking.

Aqueous Cream BP 2001 was selected as an example of an anion-active O/W emulsion, Clioquinol Cream BP 1999 without clioquinol and Nonionic Hydrophilic Cream DAB 2001 as examples of nonionic O/W emulsions with a creamy consistency, Hydrophilic Skin Emulsion Base S. 25. as an example of a nonionic O/W emulsion of the lotion type, and Base Cream DAC as an example of an ambiphilic emulsion.

4.3. Test areas

Three circular symmetrical test areas (radius 1.5 cm) on both forearms and two circular symmetrical test areas (radius 1.5 cm) on both upper arms. The test areas on one side were left untreated. The treated/untreated sides were reversed from subject to subject.
The treated test areas were marked A (distal), B, C, D, and E (proximal). The untreated test areas were marked AA (distal), BB, CC, DD, and EE (proximal). The application sites of the study emulsions were as follows:

A vs AA: Aqueous Cream BP vs untreated
B vs BB: Clioquinol Cream BP without clioquinol vs untreated
C vs CC: Nonionic Hydrophilic Cream DAB without glycerol vs untreated
D vs DD: Hydrophilic Skin Emulsion Base NRF S.25. without glycerol vs untreated
E vs EE: Base Cream DAC vs untreated

4.4. Study design

Subjects were instructed to uniformly and evenly apply 0.1 mL of each study emulsion to the designated test areas twice daily for 7 days using a tuberculin syringe and small cotton applicator. At baseline (day 0) and 12 h after the last application (day 7), TEWL, skin redness (a*-value), and erythrocyte circulation in the subpapillary vessels (laser Doppler) were determined on the treated and untreated sites.
Fifteen subjects then underwent the hydrocortisone blanching test using Hydrocortisone Cream 0.5% NRF 11.36. (hydrocortisone 0.5, medium chain triglycerides 1.5, Base Cream DAC (for composition see above) ad 100.0). 0.1 mL of Hydrocortisone Cream 0.5% NRF 11.36. was applied in a Finn Chamber (1.6 cm in diameter; Hermal Chemie, Reinbek, Germany). The Finn Chambers were attached with adhesive bandages (Cutiplast® Steril Pflaster 7.2 × 5 cm, Beiersdorff AG, Hamburg, Germany). After 24 h occlusion, the Finn Chambers were removed, carefully wiping off any residual product, and chromametry was performed again (day 8) as described above.
The remaining 14 subjects underwent the SLS irritation test. A 10 mm cotton patch soaked with 0.5% SLS (Merck, Darmstadt, Germany; homologous purity >98%) was applied in an occlusive chamber (diameter 12 mm). The occlusive chamber was secured with adhesive bandages (Cutiplast Steril Pflaster 7.2 × 5 cm, Beiersdorff, Hamburg, Germany). The SLS-soaked patches were left in place for 24 h. Thirty minutes after removing the occlusive chamber, TEWL, erythrocyte circulation in the subpapillary vessels, and skin redness were determined as described above.

4.5. Measuring methods

Barrier function was evaluated by measuring TEWL ($g/cm^2 \times h$) using a Tewameter TM 210 (Courage & Khazaka, Cologne, Germany) in accordance with applicable guidelines [31]. TEWL is considered an important measure of epidermal barrier function. Evaporimetry consists of applying a probe with two twin sensors at two levels directly to the skin, with one sensor pair measuring humidity and the other temperature. The acquired data are used by an integrated microcomputer to compute the water vapor partial pressures at the two parallel levels of each sensor pair and, via the partial pressure gradient, the rate of evaporation. To minimize outside interferences, the measurements were carried out in an open-top Plexiglas chamber with closed sides.
Skin color can be measured by tristimulus (blue, red, green) analysis of light reflected from skin using a CR 200 chromameter (Minolta, Osaka, Japan). The color of the reflected light from a pulsed xenon arc lamp was analyzed by three high-sensitivity silicon photocells filtered to match the CIE standard observer curves for the primary colors, blue (450 nm, b*-value), green (550 nm, L-value), and red (610 nm, a*-value). Higher a*-values mean greater skin redness. We therefore used the a*-value as an outcome measure for this study. Readings were relative data. The measurements were made in accordance with applicable guidelines [32].
The rate of blood (erythrocyte) flow in the capillaries was determined by measuring erythrocyte circulation in the subpapillary vessels via the Doppler effect using a PF2 Laser Doppler Flowmeter (Perimed, Jarfalla, Sweden) in accordance with applicable guidelines [33]. Readings were relative data.

4.6. Statistical analysis

TEWL, a*-value, and laser Doppler readings were analyzed for the change from baseline (day 0) to end of treatment (day 7). The hydrocortisone blanching test and SLS irritation test data were analyzed for the change from pre-test (day 7) to post-test (day 8) as well as for the change from study baseline (day 0) to study endpoint (day 8), on each occasion analyzing the differences between absolute values. The Wilcoxon Matched Pairs Signed Rank Test was used for comparison of symmetrical treated and untreated sites. Data are presented as medians, boxes (25% and 75% percentiles), minima, and maxima.

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