

## INVESTIGATIVE REPORTS

# Glycerol Accelerates Recovery of Barrier Function In Vivo\*

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Two studies were performed to evaluate the influence of glycerol on the recovery of damaged stratum corneum barrier function. Measurements of transepidermal water loss and capacitance were conducted in a 3-day follow-up after tape stripping (study 1) and a 7-day follow-up after a barrier damage due to a repeated washing with sodium lauryl sulphate. In study 1 a faster barrier repair (transepidermal water loss) was monitored in glycerol-treated sites. Significant differences between glycerol open vs. untreated and glycerol occluded vs. untreated were observed at day 3. Stratum corneum hydration showed significantly higher values in the sites treated with glycerol+occlusion, compared with all other sites. In study 2 a faster barrier repair was seen in glycerol-treated sites, with significant differences against untreated and base-treated sites 7 days after the end of the treatment. Stratum corneum hydration showed highest values in the glycerol treated sites after 3 days of treatment. Glycerol creates a stimulus for barrier repair and improves the stratum corneum hydration; stratum corneum hydration is not strictly related to barrier homeostasis and can be optimized by different mechanisms and pathways. The observed effects were based on the modulation of barrier repair and were not biased by the humectant effect of glycerol. As the glycerol-induced recovery of barrier function and stratum corneum hydration were observed even 7 days after the end of treatment, glycerol can be regarded as a barrier stabilizing and moisturizing compound. **Key words:** tape stripping; SLS washing; transepidermal water loss (TEWL); capacitance; occlusion; barrier repair.

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The mechanisms promoting barrier repair in vivo after stripping of the stratum corneum (SC) and repeated irritation with sodium lauryl sulphate (SLS) are not completely clear: the modulation of water flux is probably a key factor involved in barrier repair (1–7). It is known, that glycerol represents a hygroscopic compound capable of absorbing water from the environment and deeper parts of the SC.

The purpose of the present study was to evaluate in vivo the effects of glycerol and occlusion in the promotion of

barrier repair. Two studies were performed to evaluate the effect of a repeated application of glycerol on damaged SC barrier. The barrier disruption was performed by tape stripping (study 1) and by repeated washing with SLS over 4 days (study 2).

## MATERIALS AND METHODS

### Volunteers

Study 1 was carried out with 12 healthy female volunteers (age range 24–35 years). Study 2 was carried out with 19 healthy volunteers (19–44 years); 7 male and 12 female. Their written informed consent was obtained.

### Experimental design

#### Study 1

Two sites (2 × 2 cm) were selected on each volar forearm and were stripped approximately 15 times using an adhesive scotch tape (Scotch, USA) until a transepidermal water loss (TEWL) level of approximately 15 g/m<sup>2</sup> × h was reached. The sites were then treated for 3 days with glycerol (99.8%, Sigma, Germany), glycerol and occlusion, or occlusion alone, respectively. One site was left untreated. The amount of glycerol under the polypropylene plastic chamber (18 mm) (Hill Top<sup>®</sup>, Cincinnati, USA) was 0.1 ml once per day, and on the open site 3 times per day 0.1 ml. Before starting the measurements, the volunteers had to acclimatize for 30 min at 22 ± 1°C (40% ± 1% relative humidity). Occlusion was performed for 24 h. At the end of every 24-h period occlusion was removed and treated sites were measured after 1 h. After the measurements glycerol was applied and occlusion was reinstalled.

#### Study 2

The 4 test areas were on the proximal and distal part of the volar forearm. Before performing the washing procedure, these areas were separated by an adhesive scotch tape. The size of the treated areas were 2 × 2 cm. After taking the initial values (t0) a standardized washing procedure was performed with 2% SLS solution (Merck, Germany). A 200 g heavy foam roller was rolled 50 times over each test site. Every 10–15 times the foam rolled was dipped in the SLS solution. This standardized washing procedure was performed 3 times per day by trained volunteers during 4 days. At the end of the 4 days (t4) the TEWL values representing the barrier function had increased at all test areas from ~5 g/m<sup>2</sup> × h to ~17 g/m<sup>2</sup> × h, without a significant difference between the 4 test sites. The washing procedure was stopped at this moment. After the barrier disruption due to the repeated SLS washing a treatment with 0.2 ml (=0.05 ml/cm<sup>2</sup>) of 3 different ointments was performed during 3 days, 3 times per day. 12 h (t7) and 7 days (t14) after the last treatment measurements were performed. The 3 test formulations were composed as following: all contained 33.3% DAC base cream (amphiphilic emulsion, German pharmacopoeia; Bombastus, Germany: glycerol monostearate 60 40.0 g; cetyl alcohol 6.0 g; triglycerides 7.5 g, petrolatum 25.5 g; macrogol-1000-glycerol monostearate 7.0 g.; propylene glycol 10.0 g.; purified water 40.0 g Wasserfuhr, Germany).

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1. A: DAC+water 66.7%
2. B: DAC+glycerol 85% 25.0+water 41.7
3. C: DAC+glycerol 85% 50.0+ water 16.7

The preparation and randomization of the test formulations in study 2 was performed by Drais Pharmacy, Karlsruhe (directed by Ms Zybowski). The test sites on the ventral forearms in both studies were randomized and rotated and 1 site was left untreated.

#### Instrumental evaluation

Barrier repair was quantified by the measurement of TEWL and SC hydration by capacitance. We used the Evaporimeter EP 1 (ServoMed, Stockholm, Sweden) in study 1; the Tewameter TM 210 (Courage & Khazaka, Cologne, Germany) in study 2 and the Corneometer CM 820 (Courage & Khazaka, Cologne, Germany) in both studies. The measurements were performed according to the valid guidelines (8, 9).

#### Data handling and statistics

In study 1, raw data were baseline-adjusted by subtracting the value from day 0 after tape-stripping. Distribution normality was tested with the Kolmogorow-Smirnov-Test and the Lilliefors-Test. For each data-group (Capacitance and TEWL at each time) an analysis of variance for repeated measurements (ANOVA) was performed. The level of  $p < 0.05$  was considered significant. A post-hoc comparison between the groups was performed with the Least Significant Difference (LSD) test (study 1) and a priori-ordered hypothesis testing (Wilcoxon rank test) according to Maurer et al. (10) (study 2). Statistical analysis were performed with Statistica software package (StatSoft, USA) and Prism2 (GraphPad, USA).

## RESULTS

The results are shown in Figs 1–4.

#### Study 1

A faster decrease in TEWL values in the sites treated with glycerol vs. control sites was monitored during days 1 to 3. Statistically significant differences between glycerol-treated and control sites were observed at day 3 (glycerol open vs. untreated,  $p < 0.04$  and glycerol occluded vs. untreated

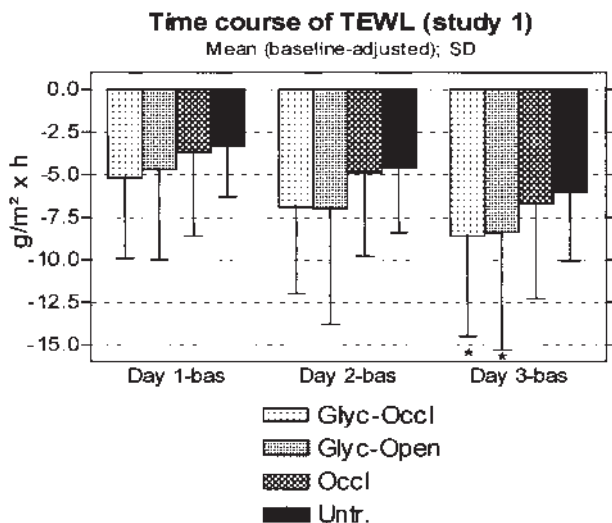


Fig. 1. Transepidermal water loss (study 1) in a 3-day follow-up after tape stripping. Mean values (baseline-adjusted) and SD. \*Significant vs. untreated.

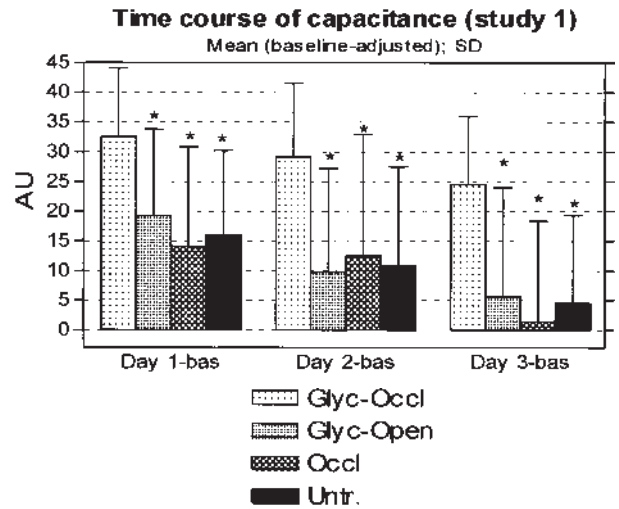


Fig. 2. Capacitance values (AU) in a 3-day follow-up after tape stripping. Mean values (baseline-adjusted) and SD. \*Significant vs. glycerol occlusion.

$p < 0.03$ ). Occlusion alone did not result in any significant difference vs. untreated skin. SC hydration showed significantly higher values ( $p < 0.001$ ) in the sites treated with glycerol and glycerol+occlusion as compared to the other test sites during the entire study (Figs. 1–2).

#### Study 2

A marked barrier damage due to the repeated SLS washing (increased TEWL) as well as a SC dehydration (decreased capacitance) was detectable at t4 (Figs. 3–4). After 3 days of treatment, lower TEWL values and elevated capacitance values were seen in the treated sites in comparison with the untreated area. The ANOVA for the TEWL values at t7 was not significant, ( $p = 0.1932$ ), while the ANOVA was significant for the capacitance ( $p < 0.0001$ ). Both glycerol-treated sites

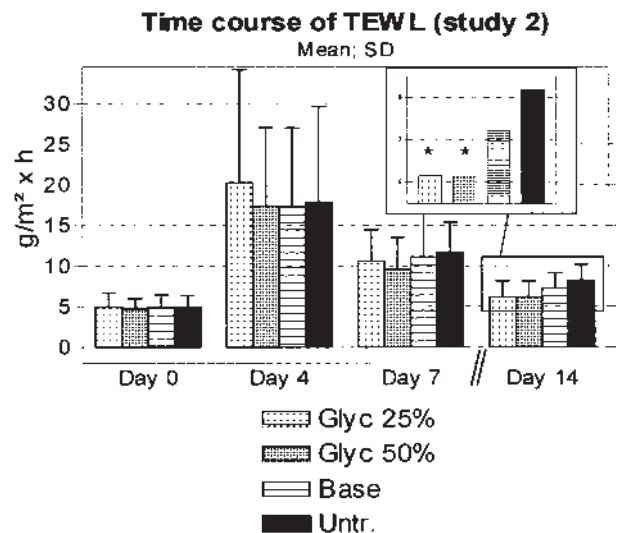


Fig. 3. Transepidermal water loss (study 2) at t0, after 4 days (t4) of repeated SLS washing, 3 days (t7) of treatment and a 7-day follow-up (t14). Mean values and SD. Values from day 14 were enlarged for a better illustration. \*Significant vs. base.

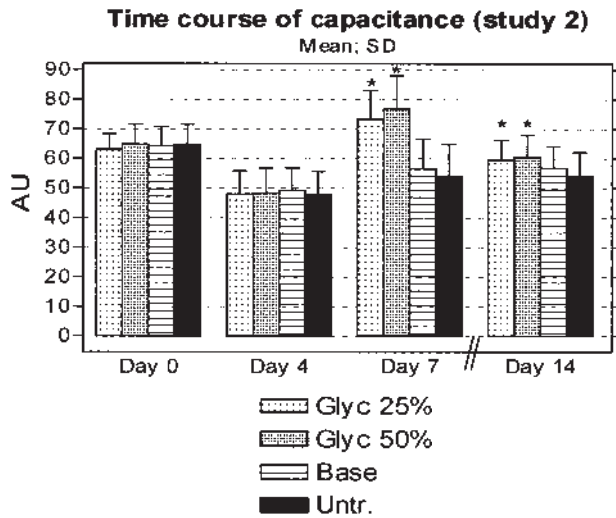


Fig. 4. Capacitance values (AU) at t0, after 4 days (t4) of repeated SLS washing, 3 days (t7) of treatment and a 7 days follow-up (t14). Mean values and SD. \*Significant vs. base.

showed the highest values with significantly different values against untreated and base-treated test sites ( $p < 0.001$  for all differences). The ANOVA for the follow-up after 7 days without any treatment (t14) was significant for the TEWL values ( $p < 0.0001$ ) and for the capacitance values ( $p = 0.0012$ ). The lowest TEWL values were seen in the 2 glycerol-treated sites with a significant difference against the untreated area ( $p < 0.001$ ). The stratum corneum hydration was highest in the 2 glycerol-treated sites with significant differences against the untreated and base-treated test area (both:  $p < 0.01$ ) (see Figs. 3–4).

## DISCUSSION

The most important pharmacological properties of glycerol have been reviewed by Gloor et al. (11). These are: hydration of the SC, especially in emulsion systems (12, 13); hygroscopicity (13, 14); keratolytic effect by desmosome degradation (15); smoothing effects (12, 16, 17); and protective function in emulsion-systems against irritations (18). Furthermore, it is known that glycerol acts as a moisturizer by absorbing water. Glycerol, by its hygroscopic property, is able to bind water and to lower water evaporation (19). It has been shown that the skin-moisturizing effect depends on the amount of absorbed humectant and on the physico-chemical properties in the stratum corneum (20). The mechanisms promoting barrier repair in vivo after barrier disruption are not completely clear. Tape stripping is known to lead to a proliferative stimulus and hyperproliferation after removing layers of stratum corneum (21). TEWL remains elevated some days after tape stripping and SLS irritation. Repair mechanisms lead to a normalization of TEWL values. The modulation of water flux after tape stripping is probably a key factor involved. Studies on hairless mice showed the lipid synthesis and hyperproliferation for barrier recovery after disturbance was delayed under occlusion (2, 3). In human volunteers after barrier damage (acetone, tape-stripping, SLS), occlusion delayed the normalization of TEWL (4). In contrast, other groups showed no effects of occlusion on barrier recovery in

humans (5–7). An improvement of barrier function by glycerol under occlusion after tape-stripping was observed by measuring the alkali resistance and the irritant effect of dimethylsulphoxide (1).

Study 1 showed an improvement of barrier function from day 1 to 3 after stripping in the sites treated with glycerol open and glycerol+occlusion. No significant difference between the open and occluded application of glycerol could be detected on TEWL-values. Differences were seen between both glycerol-treated sites and the untreated sites, at day 3 ( $p < 0.03$  and  $p < 0.04$ ). Glycerol+occlusion-treated site was, in all 3 days, significantly more hydrated than the other 3 sites. No significant differences were found between occluded and untreated sites regarding TEWL and capacitance values. Therefore, occlusion alone did not result in changes in barrier repair and SC hydration, as shown in other studies performed in different experimental conditions (1, 5, 7). On the other hand, occlusion+glycerol were capable to enhance the moisturizing properties of the system (by providing extra water for binding with glycerol), but did not influence the water flux modulation through the deeper layers of the SC and therefore barrier repair induced by glycerol itself.

Study 2 was performed since the influence of the physical properties of glycerol on TEWL values (due to the prolonged water absorption (14)) in study 1 could not be excluded. The recovery of barrier function was seen in both glycerol-treated groups independently of the glycerol concentration. This difference was statistically significant 7 days after the end of the treatment.

In conclusion, glycerol, by absorbing water can stimulate a water flux creating a stimulus for barrier repair. The repair stimulus of glycerol on barrier function was not decreased under occlusion. The SC hydration is not strictly related to barrier homeostasis and can be reached by different mechanisms and pathways. Indeed, the combination of glycerol+occlusion showed the most powerful, statistically significant hydrating effect. But the effect of glycerol+occlusion on barrier repair was not superior to the effect of glycerol alone.

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