**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 (transpidermal 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 x 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/m² x 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®, Cincinnati, USA) was 0.1 ml once per day, and on the open site 3 times per day 0.1ml. 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 x 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/m² x h to ~17g/m² x 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/cm²) 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; cetlyl alcohol 6.0 g; triglycerides 7.5 g, petrolatum 25.5 g; macro-gol-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

![Time course of TEWL (study 1)](image)

*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.*

$<0.03$). 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

![Time course of TEWL (study 2)](image)

*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.*
Groups showed no effects of occlusion on barrier recovery in delayed the normalization of TEWL (4). In contrast, other was delayed under occlusion (2, 3). In human volunteers after disturbance involved. Studies on hairless mice showed the lipid synthesis of water flux after tape stripping is probably a key factor. Let's lead to a normalization of TEWL values. The modulation repair mechanisms and pathways. Indeed, the combination of barrier homeostasis and can be reached by different 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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