

The efficacy of different moisturizers on barrier recovery in hairless mice evaluated by non-invasive bioengineering methods

A model to select the potentially most effective product

CHARLOTTE G. MORTZ¹, KLAUS E. ANDERSEN¹ AND LARS HALKIER-SØRENSEN²

¹Department of Dermatology, Odense University Hospital, DK-5000 Odense C, Denmark

²Department of Dermatology, Marselisborg Hospital, University of Aarhus, DK-8000 Aarhus C, Denmark

Moisturizers (emollients) are used frequently on normal and diseased skin. However, only few studies have examined their effects in dynamic situations and in more clinically relevant settings. We evaluated the effect of 4 commonly used products in a hairless mice model after acute skin barrier perturbation with acetone. The efficacy was evaluated by measurement of the transepidermal water loss (TEWL) and electrical conductance at various time intervals during barrier repair. The test products were compared with acetone-treated air-exposed controls allowed to recover otherwise normally and with a known irritant product, chlorhexidine cream 1%. Locobase® was the most effective product in correcting barrier function and significantly improved barrier function during early stages of barrier recovery (<6 h) without interfering with late stages of barrier recovery (>6 h). The irritant control product, chlorhexidine cream 1%, delayed barrier recovery in the late stages. The model makes it possible to evaluate the combined effects of exogenous and endogenous components on barrier repair and to select the potentially most effective products before performing more cumbersome and time-consuming field studies.

Key words: emollients; moisturizers; chlorhexidine; skin barrier; transepidermal water loss (TEWL); electrical conductance; skin temperature; hairless mice; dermatitis; prevention; therapy.
© Munksgaard, 1997.

Accepted for publication 28 February 1997

Moisturizers (emollients) are recommended for dry and diseased skin, and they are frequently used in dermatology and among workers in different industries. Previous studies have documented the efficacy of moisturizers using non-invasive bioengineering methods in healthy volunteers with normal skin (1-4). However, until recently only few studies of moisturizers have focused on their efficacy and mechanism of action in the epidermis after various types of acute or chronic skin damage or during everyday exposure to various irritants, representing the more clinically relevant settings in which the products are used. Because the products may interfere with various types of skin reactions that occur after skin damage, i.e. barrier repair, conditions drawn from normal skin cannot without reservation be applied to damaged skin. The present study evaluates the efficacy of 4 commercially available and commonly used moisturizers in a hairless mouse model after

barrier perturbation with acetone. To investigate whether the moisturizers possessed irritant properties, we included a known irritant product, chlorhexidine cream 1%, as a positive control (5). Cutaneous barrier function was assessed by measurements of the transepidermal water loss (TEWL) and electrical conductance. The results were compared with acetone-treated air-exposed controls allowed to recover otherwise normally. Acetone was used, because it selectively removes the stratum corneum lipids, giving minimal cytotoxicity and a well-described response (6).

Materials and Methods

Animals

Hairless, female mice (HR/HR) 10-19 weeks old were purchased from Bomholtgaard, Denmark. They were fed a mouse diet (Atromin number

1324, Brogaarden, Denmark), water ad libitum, and caged with a 12 h day/night cycle. Each product was tested in parallel in 10 mice, and a total of 22 control mice were included. Mice treated with moisturizers or chlorhexidine cream 1% were compared only with acetone-treated air-exposed controls within the same group and within the same period of time, to minimize variation between groups of animals and day-to-day variation (7). The study was performed blinded to avoid bias.

Anesthesia

The animals were anaesthetized by intraperitoneal injection of 0.005 ml/g of Hypnorm® (fluanisone 10 mg/ml, fentanyl 0.2 mg/ml, JanssensPharma A/S, Denmark), Apozepam® (5 g/ml, Apotekernes Laboratorium, Norway) and isotonic NaCl (1:1:2) before barrier disruption and application of moisturizers or Chlorhexidine cream 1%. At later time points intraperitoneal injection of 0.005 ml/g Sombrevin® (propanidid, Lenau & Co, Denmark) was used for short-term anesthesia.

Products tested

Essex® (Schering Plough, Denmark), Decubal® (Dumex A/S, Denmark), Locobase® (Yamanouchi Pharma A/S, Denmark) and Natusan® (Johnson & Johnson A/S, Denmark) were selected, because they are often recommended in Denmark and previously studied in normal human skin (7).

Chlorhexidine cream 1% (DAK, Denmark) was used as an irritant control product.

Skin physiological measurements

Transepidermal water loss (TEWL) (Evaporimeter EP1, ServoMed, Stockholm, Sweden), electrical conductance (Skicon 200, IBS Ltd., Tokyo, Japan) and skin surface temperature (Digital thermometer, Ellab type TRD, probe diameter 12 mm) were measured on the flanks of the mice. The probes were handheld using gloves. Transepidermal water loss (TEWL) and skin surface temperature were measured once. Electrical conductance was measured 2× at 5 points and expressed as a median. All measurements were performed in an incubator to minimize environmental disturbances and to minimize draught. Anaesthetized animals were kept warm by an incandescent bulb to maintain a normal body temperature.

Barrier disruption and applications

To perturb the barrier function the flanks were gently treated with acetone-soaked cotton balls

(Bie & Berntsen) as described previously (8). Transepidermal water loss (TEWL) levels were increased from 10 (8–10) g/m²h to 52 (46–60) g/m²h (medians and 25–75 percentiles). A standardized amount of moisturizer or chlorhexidine cream 1% was subsequently applied to an area of 1.6 cm² for 1 min with a gloved finger. New gloves were used before each application. In case of remnants of the products on the skin surface, 10 min before the first measurement they were removed with absorbent paper tissue. Measurements were performed at various time intervals (1, 2, 3, 6, 9 and 24 h) at treated sites and on normal skin on the contralateral site. The barrier recovery in mice treated with moisturizers or chlorhexidine cream 1% were compared with acetone-treated air-exposed controls allowed to recover otherwise normally.

Recovery of the transepidermal water loss (TEWL) and conductance was calculated using the equation:

$$(1 - (x_t - \text{basal value}) / (x_0 - \text{basal value})) \times 100$$

x_t = reading of TEWL or conductance at t ; x_0 = reading of TEWL or conductance immediately after barrier disruption; $t = 1, 2, 3, 6, 9, 24$ h.

Statistical methods

Results are given as medians and 25–75 percentiles. Statistical analyses of data at various time points were performed by using the non-parametric Kruskal-Wallis test, Mann-Whitney U -test and Spearman's rank correlation coefficient ($p < 0.05$).

Results

The skin surface temperature of the mice was constant during the trial, so we did not correct the transepidermal water loss (TEWL) for temperature. Measurement of TEWL and conductance on normal skin were also constant during the trial. Room temperature was 22.7°C (21.3–25.0°C) and relative humidity (RH) 51% (37–66%).

Fig. 1 shows the barrier recovery for the different products versus acetone-treated air-exposed controls. Locobase® significantly improved barrier recovery during early stages (<6 h) and did not interfere with the barrier recovery in late stages (>6 h) (Fig. 1 A). Essex® significantly improved barrier recovery after 1 h, but after 24 h Essex® slightly delayed barrier recovery (Fig. 1B). Natusan® showed no effect on barrier recovery either in the early phase or in the late phase (Fig. 1C). Decubal® had no effect in the early stages, but in the late stages it delayed barrier recovery signifi-

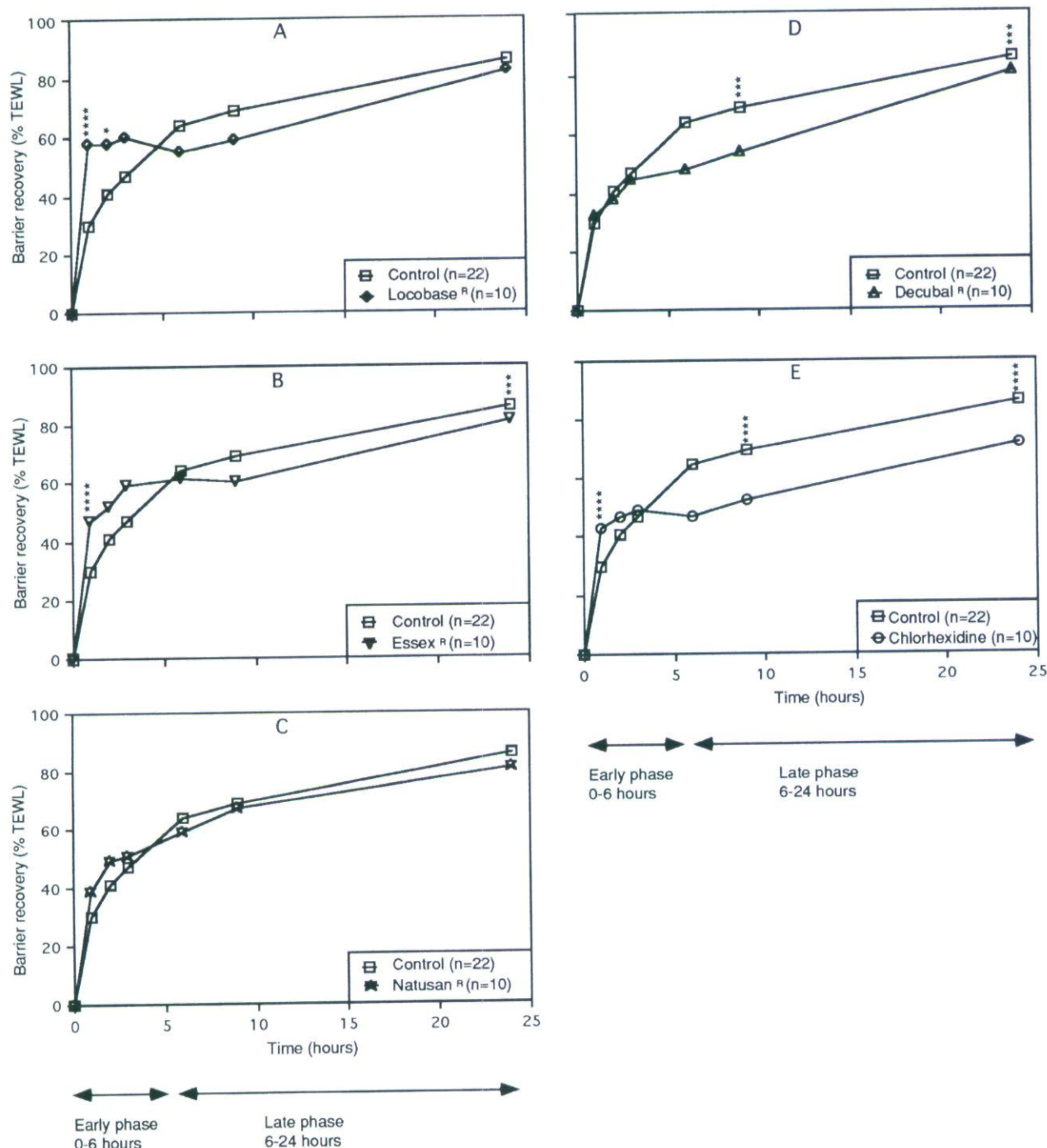


Fig. 1. The effect of Locobase cream®, Essex cream®, Natusan cream®, Decubal cream® and chlorhexidine cream 1% on barrier recovery (% TEWL) compared to acetone-treated air-exposed controls. Data points represent medians. Mann-Whitney U-test: * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.005$, **** $p \leq 0.001$.

cantly (Fig. 1D). Chlorhexidine cream 1% significantly improved barrier function after 1 h, but in the late stages it significantly delayed barrier recovery (Fig. 1E).

Fig. 2 shows the relationship between recovery of transepidermal water loss (TEWL) and conductance after 1 h for the 3 products that differed sig-

nificantly from acetone-treated air-exposed controls (Spearman's rank correlation coefficient, $p < 0.05$). Locobase® caused the most pronounced improvement in barrier function (lowest TEWL, highest conductance), whereas chlorhexidine cream 1% was associated with the highest TEWL and lowest conductance.

	TEWL	Conductance
Locobase [®] cream	S	S
Essex [®] cream	S	S
chlorhexidine cream 1%	S	S
Natusan [®] cream	NS	NS
Decubal [®] cream	NS	NS

S: Significant from acetone treated air-exposed controls.

NS: Non-significant from acetone treated air-exposed controls.

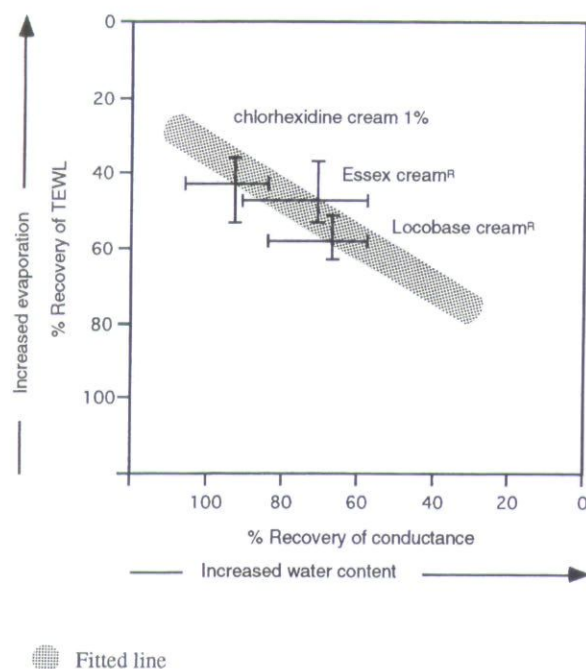


Fig. 2. Relationship between TEWL and conductance for Locobase cream[®], Essex cream[®] and chlorhexidine cream 1% after 1 h. Natusan[®] and Decubal[®] were non-significant from acetone treated air-exposed controls. Data points represent medians and 25–75 percentiles. Spearman's rank correlation coefficient: $p \leq 0.001$.

Discussion

The present animal model was used to investigate whether moisturizers (emollients) affect barrier function in dynamic situations and to discriminate between the effects of different products on barrier recovery.

In a previous study, it has been shown that Locobase[®] increased the skin hydration (capacitance) in normal skin in healthy volunteers (7). This study demonstrated that Locobase[®] was the most effective product in correcting barrier function (transepidermal water loss (TEWL)) in damaged murine skin. Locobase[®] significantly improved barrier function during the early stages (<6 h) of barrier recovery without interfering with endogenous barrier recovery at later stages (>6 h) (Fig. 1A). Furthermore, the efficacy of Locobase[®] has been tested among cleaners and kitchen workers during

everyday exposure to various irritants, and a positive effect was demonstrated both by clinical assessment and skin physiological measurements (capacitance) (9). Field studies are important before recommending moisturizers to workers, because it has been shown that moisturizers and various lipid components may interact both with barrier recovery and various industrial products (10–15). None of the products in this study impeded barrier recovery in the early stages (<6 h), but 2 of the 4 moisturizers significantly delayed barrier recovery in the late stages (>6 h), and they may therefore interfere with endogenous lipid synthesis (Fig. 1B, 1D). However, the clinical relevance may be questionable and should be tested. Chlorhexidine cream 1% is a known irritant in the chamber scarification test (5), and in our study it caused a more pronounced delay in barrier recovery in the late stages compared to acetone-treated air-exposed controls (Fig. 1E). The deteriorating effect on barrier recovery seen in this study may be explained by its irritant properties. Not only the irritant properties of a product but also occlusion may cause delay in barrier recovery. It has been shown that a vapor-impermeable membrane (Latex[®]) delayed barrier recovery after barrier perturbation with acetone in hairless mice, whereas a vapor-permeable one (Goretex[®]) did not (8). However, the effect of occlusion on barrier recovery in human skin is questionable (16). Furthermore, although petrolatum is one of the most greasy and occlusive skin care products, petrolatum did not delay barrier recovery after barrier perturbation with acetone, either in mice or in humans (17). Therefore moisturizers are probably only semioclusive, and another mechanism may account for the delay in later stages.

When measurements of the transepidermal water loss (TEWL) and conductance were compared for the 3 products that significantly changed both TEWL and conductance, we found a positive correlation between TEWL and conductance after 1 h (Fig. 2). Locobase[®] caused the most pronounced improvement in barrier function (lowest TEWL, highest conductance), whereas chlorhexidine cream 1% was associated with the highest TEWL and lowest conductance (positive control).

Studies in healthy volunteers (basal conditions) have shown that application of moisturizers on normal skin has an effect on electrical conductance/capacitance, but not on transepidermal water loss (TEWL) (1, 2, 7). Using the dynamic model, we found that some products significantly improved barrier recovery evaluated by measurement of TEWL. One of those products was Locobase[®]. Therefore Locobase[®] was selected and evaluated in a field study among cleaners and kitchen

workers. The field study demonstrated that cleaners and kitchen workers benefit from the use of Locobase[®], evaluated by both clinical assessment and skin hydration (capacitance). However, no change in TEWL was found in this study design, perhaps because the measurements were performed on well-defined areas and not in skin with visible changes (9).

The animal model presented makes it possible to select the potentially most effective products before performing more cumbersome and time-consuming field studies. Because the time courses for barrier recovery for animals and humans are different, it is not advisable to transfer results from animal studies directly to human situations. However, a similar, parallel and significant improvement in barrier recovery has been found during petrolatum treatment after barrier perturbation with acetone in mice and humans (9). Further studies are needed to show whether results from this model can in general be transferred to human situations.

Acknowledgements

The study was supported by P. Carl Petersens Foundation, Copenhagen, Denmark, Institute of Clinical Research, Odense University Hospital, Denmark and Yamanouchi Pharma A/S, Denmark. We thank Dumex A/S, Denmark and J. Serup, Leo Pharmaceutical products Ltd. A/S, Denmark for lending us the Skicon 200.

References

1. Blichmann C W, Serup J, Winther A. Effects of single application of a moisturizer: evaporation of emulsion water, skin surface temperature, electrical conductance, electrical capacitance and skin surface (emulsion) lipids. *Acta Dermato-venereologica* 1989; 69: 327-330.
2. Serup J, Winther A, Blichmann C W. Effect of repeated application of a moisturizer. *Acta Dermato-venereologica* 1989; 69: 457-459.
3. Korstanje C, Ligtoet F P T, Van Hemert K H F. Differential effects of dermatological cream bases with respect to skin surface moisturizing capacity: a study design in volunteers. *J Dermatol Treat* 1992; 2: 137-139.

4. Grove G L. The effect of moisturizers on skin surface hydration as measured in vivo by electrical conductivity. *Current Therapeutic Research* 1991; 50: 712-719.
5. Andersen K E. Reproducibility of the chamber scarification test. *Contact Dermatitis* 1996; 34: 181-184.
6. Menon G K, Feingold K R, Moser A H, Brown B E, Elias P M. De novo sterogenesis in the skin. II. Regulation by cutaneous barrier requirements. *J Lipid Res* 1985; 26: 418-427.
7. Halkier-Sørensen L. Occupational skin diseases. *Contact Dermatitis* 1996; suppl 1: 1-120.
8. Grubauer G, Elias P M, Feingold K R. Transepidermal water loss: the signal for recovery of barrier structure and function. *J Lipid Res* 1989; 30: 323-334.
9. Halkier-Sørensen L, Thestrup-Pedersen K. The efficacy of a moisturizer (Locobase) among cleaners and kitchen assistants during everyday exposure to water and detergents. *Contact Dermatitis* 1993; 29: 1-6.
10. Mann M Q, Feingold K R, Elias P M. Exogenous lipids influence permeability barrier recovery in acetone-treated murine skin. *Arch Dermatol* 1993; 129: 728-738.
11. Goh C L, Gan S L. Efficacies of a barrier cream and an afterwork emollient cream against cutting fluid dermatitis in metalworkers: a prospective study. *Contact Dermatitis* 1994; 31: 176-180.
12. Frosch P, Schulze-Dirks A, Hoffmann M et al. Efficacy of skin barrier creams (I). The repetitive irritation test (RIT) in the guinea pig. *Contact Dermatitis* 1993; 28: 94-100.
13. Frosch P, Schulze-Dirks A, Hoffmann M, Axthelm I. Efficacy of skin barrier creams (II). Ineffectiveness of a popular "skin protector" against various irritants in the repetitive irritation test in guinea pig. *Contact Dermatitis* 1993; 29: 74-77.
14. Frosch P, Kurte A, Pilz B. Efficacy of skin barrier creams (III). The repetitive irritation test (RIT) in humans. *Contact Dermatitis* 1993; 29: 113-118.
15. Frosch P, Kurte A. Efficacy of skin barrier creams. (IV). The repetitive irritation test (RIT) with a set of 4 standard irritants. *Contact Dermatitis* 1994; 31: 161-168.
16. Welzel J, Wilhelm K P, Wolff H H. Skin permeability barrier and occlusion: no delay of repair in irritated human skin. *Contact Dermatitis* 1996; 35: 163-168.
17. Ghadially R, Halkier-Sørensen L, Elias P M. Effects of petrolatum on stratum corneum structure and function. *J Am Acad Dermatol* 1992; 26: 387-396.

Address:

Charlotte G. Mortz
Department of Dermatology
Odense University Hospital
DK-5000 Odense C
Denmark

E-mail: mortz@imbmed.ou.dk.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.