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Measurement of Sodium Lauryl Sulfate-induced Skin Irritation

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Besides visual evaluation, skin irritation induced by sodium lauryl sulfate (SLS) may be characterized by bioengineering measurements, such as skin colour reflectance, transepidermal water loss (TEWL) or hydration. Short application times or low concentrations of the irritant usually do not modify the visual aspect of the skin, and the measurements described above are unchanged or only slightly altered.

We were looking for a suitable method to measure cutaneous changes not detectable by usual bioengineering procedures. Therefore these measurements were compared to those of dynamic function testing of the stratum corneum, namely sorption-desorption and moisture accumulation tests. Different concentrations of SLS (0.1%, 0.5%, 2.5%), application times (15 min, 24 h) and times of testing (1 h, 24 h after patch removal) were investigated on the ventral forearm of human subjects. When SLS was applied for a short period (15 min), 1 h after patch removal skin colour, TEWL and hydration were not modified, while increases in hygroscopicity, water-holding capacity and water accumulation were detected depending on the applied concentration. Increase of hygroscopicity was closely correlated with the alteration of epidermal barrier function (TEWL).

We demonstrated that sorption-desorption and moisture accumulation tests performed on SLS-treated areas for a short period, without visible modifications, could evaluate changes of the stratum corneum properties. We consider these tests as useful complementary methods to skin colour, TEWL and hydration measurements, particularly in the detection of subclinical skin injuries. ***Key words: human skin; stratum corneum; hydration; transepidermal water loss.***

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Sodium lauryl sulfate (SLS) is a common surfactant, which is acknowledged as a reference irritant (1–3). It is well suitable for irritant patch testing because of its ability to influence the barrier function of the skin and to cause inflammation. A linear dose-response relationship between dose of SLS and skin response is seen using visual score or bioengineering techniques, such as measurements of skin colour, transepidermal water loss (TEWL) and superficial blood flow (3, 4). Short application times (1–10 min) induce transient increases (during 10–15 min after patch removal) of skin hydration and skin surface water loss measurements (5). After this time, measured parameters returned to normal. This does not necessarily imply that the state of the challenged skin has returned to normal. Further, evidence is growing that non-invasive methods will gain more importance in the near future because of their practical relevance in dermatology (6). The prohibition of animal experimentation for cosmetic products in the EC after January the 1th 1998 (7) will also contribute to the increased use of these techniques on human volunteers. The aim of our study was to compare results obtained by measurements of skin colour, TEWL and hydration with those of dynamic function testing such as the sorption-desorption (8) (SDT) and the moisture accumulation (9) (MAT) tests after SLS application. Both tests measure skin response to an external stimulus, whereas other techniques investigate the state of the skin at a given time point. External stimuli are water and occlusion for SDT and MAT, respectively. To cover a range of experimental conditions, different SLS concentrations (0–2.5%), application times (15 min, 24 h) and times of testing (1 h, 24 h after patch removal) were investigated.

MATERIALS AND METHODS

Ten healthy volunteers (4 females and 6 males), aged 27–49 years; 34.5 ± 8.1, participated in the study. Informed consent was obtained from all participants. The investigations were conducted between February and May 1994 in an air-conditioned room (temperature 22 ± 1°C, relative humidity 45 ± 5%). Subjects rested for 20 min before measurements. The ventral side of the forearm was chosen as test region. Closed patch tests (Finn chambers of diameter 12 mm and corresponding filter discs) with 50 µl of aqueous solutions (weight/volume) of 0 (control), 0.1, 0.5, 2.5% SLS (98% purity, Fluka Chemie, Buchs, Switzerland) were randomly applied onto the forearms. Test chambers were removed after either 15 min or 24 h and measurements were performed either 1 h or 24 h after removal, respectively.

Skin colour reflectance

Skin colour was measured by a Chromameter CR 300 (Minolta, Osaka, Japan) calibrated to standard white and orange plate at the beginning of each measurement day. This device uses the three-dimensional coordinate system of the Commission Internationale de l'Eclairage with a brightness axis (L*), a green-red axis (a*) and a blue-yellow axis (b*). Observations on the green-red axis (redness of the skin) were used (3, 4).

Transepidermal water loss (TEWL)

TEWL was measured using the evaporimeter EP1 (Servo Med, Stockolm, Sweden) according to the guidelines of the Standardization Group of the European Society of Contact Dermatitis (10).

Hydration

The hydration level was measured by a Nova DPM 9003, equipped with a sensor probe DPM 9103 (Nova, Gloucester, MA), of which the measuring principle has previously been described (11). Briefly, this device integrates selected measurements at various frequencies of an applied alternating current and takes a number of samples along a controlled time rise up to 1 MHz. The final readout is given in arbitrary units, which are related to the capacitance. The measurements were shown to be reliable in detecting hydration changes of the stratum corneum (11).

Sorption-desorption test (SDT)

The SDT was performed as described by Tagami et al. (8), with the Nova DPM switched to measurement mode dl0 (11, 12). With this function setting, the reading is instantaneous when the sensor touches the skin. A first measurement was taken before patch application and another one after the application. This last value represented the start value of hydration. Then, 50 µl of distilled water were pipetted onto the skin for 10 s wiped with a paper towel and the hydration was

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immediately measured. This represented the hygroscopicity. Further measurements were performed at 0.5, 1, 1.5 and 2 min. The area under the effect curve (AUEC) resulting from these 4 time points and calculated with the trapezoidal method was defined as the water-holding capacity of the stratum corneum.

Moisture accumulation test (MAT)

The MAT was run as described by Van Neste (9) with modifications. After measuring hydration levels as described above, the Nova DPM was switched to the measurement mode Con (11, 12), allowing continuous reading, and the sensor probe was maintained on the skin for 3 min. This created occlusive conditions. Readings were recorded every 0.5 min. The AUEC (calculated as before) between start value and 3 min was defined as the water accumulation.

Statistics

The data were analysed using SPSS-PC +Statistics 4.0 software (SPSS Inc, Chicago, IL). Each parameter was tested for each application time and for each time of testing by oneway variance analysis.

RESULTS

The 15-min patch application did not cause any significant changes in skin colour, TEWL or hydration for either SLS concentration or time of testing (data not shown). A dose-dependent trend to lower hydration values was noted 1 h after patch removal (0% value = 97.0 ± 1.3 and 2.5% value = 95.0 ± 0.8; mean DPM units ± SEM). On the other side, sorption-desorption and moisture accumulation test parameters were dose-dependently increased when the measurements were performed 1 h after patch removal (Table I). These changes were less pronounced or missing 24 h after patch removal.

With the 24-h SLS patch time, significant dose-related increases were noted 1 h and 24 h after patch removal for skin colour and TEWL (data not shown). Hydration levels were significantly decreased in a dose dependent manner 1 h after patch removal (0% value = 100.0 ± 1.9 and 2.5% value = 92.5 ± 0.8; mean DPM units ± SEM) but returned to control values 24 h after patch removal. Table II indicates the results of the sorption-desorption and moisture accumulation tests for an SLS application time of 24 h. Except for hygroscopicity and water-holding capacity measured 24 h after patch removal, the SLS concentration effect was found significant.

Hygroscopicity measurements (sorption-desorption test) performed 1 h after an SLS application of 15 min were closely correlated ($r = 0.9785$, $p < 0.05$)) with TEWL measurements taken 24 h after an SLS application of the same concentrations for 24 h (Fig. 1).

Table I. *Measurements performed after an SLS patch application for 15 min*

Sorption-desorption (Hs = hygroscopicity [DPM units); WHC = water-holding capacity [DPM units. min]) and moisture accumulation (WA = water accumulation [DPM units. min]) tests were performed 1 h and 24 h after patch removal. Means ± SEM are indicated.
* significant dependence on the SLS concentration ($p < 0.001$).

SLS application for 15 min					
Parameter	Testing	0%	0.1%	0.5%	2.5%
Hs	1 h*	356 ± 28	374 ± 32	569 ± 16	701 ± 15
	24 h	473 ± 31	443 ± 43	493 ± 33	521 ± 30
WHC	1 h*	157 ± 3	153 ± 2	175 ± 10	375 ± 77
	24 h	185 ± 11	176 ± 9	182 ± 13	194 ± 25
WA	1 h*	396 ± 13	366 ± 13	453 ± 28	690 ± 64
	24 h	495 ± 40	479 ± 40	458 ± 31	443 ± 35

Table II. *Measurements performed after an SLS patch application for 24 h*

Sorption-desorption (Hs = hygroscopicity; WHC = water-holding capacity) and moisture accumulation (WA = water accumulation) tests were performed 1 h and 24 h after patch removal. Means ± SEM are indicated.
* significant dependence on the SLS concentration ($p < 0.001$).

SLS application for 24 h					
Parameter	Testing	0%	0.1%	0.5%	2.5%
Hs	1 h*	444 ± 33	361 ± 49	643 ± 36	747 ± 38
	24 h	516 ± 25	521 ± 43	577 ± 41	652 ± 37
WHC	1 h*	164 ± 5	153 ± 5	201 ± 28	423 ± 133
	24 h	185 ± 11	176 ± 9	182 ± 13	194 ± 25
WA	1 h*	500 ± 45	456 ± 61	818 ± 167	801 ± 169
	24 h*	576 ± 60	640 ± 124	872 ± 175	1002 ± 217

DISCUSSION

We measured at different time points several degrees of cutaneous irritation induced by SLS. Skin colour, TEWL and hydration were evaluated parallelly to the dynamic function testing of the stratum corneum such as sorption-desorption and moisture accumulation tests. Different SLS concentrations applied for 15 min did not significantly influence skin colour, TEWL or hydration. Use of SLS as a model irritant must therefore be performed for a longer time, usually several hours, if one considers a single occlusive application (1–3). On the other side, the results of the sorption-desorption and moisture accumulation tests were greatly modified 1 h after the patch time of 15 min. This is in agreement with a previous report (12), showing that despite normal barrier function and hydration level, other parameters such as hygroscopicity, water-holding capacity and water accumulation were significantly modified. These alterations were revealed by the external stimuli (water and occlusion) applied on the skin surface. Twenty-four hours after the 24-h patch application time,

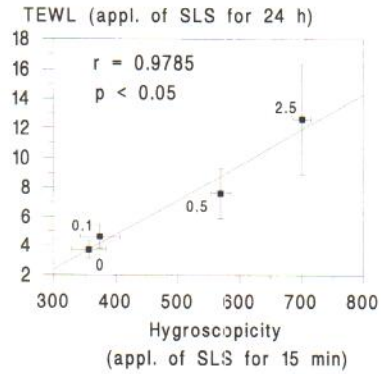

Fig. 1. Relationship between hygroscopicity values (sorption-desorption test) measured 1 h after an SLS application of 15 min and TEWL ($g/m^2.h$) values taken 24 h after an SLS application of 24 h. Means ± SEM are indicated and the numbers in the graph represent the SLS concentrations.

erythema and disrupted barrier function were present, but hygroscopicity and water-holding capacity appeared to return to normal.

In view of these findings, it may be postulated that the dynamic tests mainly evaluate some properties of the superficial stratum corneum layers that might be rapidly modified by the irritant after skin contact. It is surprising that SLS presents identical characteristics to moisturizers, as previously noticed (12), giving in a transient way the stratum corneum more capacity to take up and bind water. A disruption of the secondary and tertiary structure of keratin proteins of the skin surface, exposing new water-binding sites, was proposed as an explanation of these rapidly induced phenomena (5). Interactions with intercellular stratum corneum lipids (13–15) may also act on the stratum corneum dynamic properties, allowing the water to more easily penetrate into the stratum corneum. The relationship we found between stratum corneum hygroscopicity and TEWL measurements indicates that the ability of the stratum corneum to take up water, after a short SLS application, could predict a skin barrier damage as measured after a 24-h application of the same concentrations. Further studies are necessary to see if this holds for other irritants as well.

A similar approach to the tests described in this study has already been investigated (16), by using the plastic occlusion stress test (POST) to detect subclinical irritation. Compared with the SDT and MAT, the POST is more laborious to perform, since it requires 24-h occlusion and, as stated by the authors themselves, the interpretation of the results is rendered difficult by the superposition of skin surface water loss and TEWL. Furthermore, regarding the high SLS concentration, the repetitive applications used and the skin response, the POST seems to be less sensitive.

In conclusion, sorption-desorption and moisture accumulation tests can detect phenomena occurring within the superficial part of the stratum corneum which are not measurable by skin colour, TEWL or hydration. In view of the future regulations about product testing, their use should be helpful for dermatological or cosmetic investigations (7). We consider these tests as complementary methods to conventional skin colour, TEWL and hydration measurements, particularly in the detection of subclinical skin injuries and possibly in the prediction of irritation potential.

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