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Cumulative irritancy in man to sodium lauryl sulfate: The overlap phenomenon

S.M. Patil, P. Singh, H.I. Maibach *

Department of Dermatology, School of Medicine, University of California-San Francisco, San Francisco, CA 94143-0989, USA

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Abstract

Repeated contact of skin with surfactants causes irritation often characterized by dryness, erythema and scaliness. Morphologically healed skin after preexposure can still evoke a stronger reaction to any subsequent exposure of an irritant. Cumulative irrtancy using the overlap phenomenon was studied. The effects of repeated and prolonged application of sodium lauryl sulfate (SLS) on human skin in vivo (healthy adult volunteers) were evaluated using non-invasive techniques of TEWL and skin capacitance measurements and visual scoring. The inability of TEWL and skin capacitance to achieve pretreatment values 2 weeks post SLS treatment suggests the possible detrimental effects of SLS on skin lipids. The surfactant effect was cumulative and dehydrating effects on prolonged and repeated application were observed.

Key words: Sodium lauryl sulfate; Hydration; Cumulative irritation; Water loss

1. Introduction

The model irritant, sodium lauryl sulfate (SLS), is a standard emulsifier widely used in drug formulations, skin care and industry. Since assessment of primary skin irritation is a fundamental part of safety evaluation of topicals, many studies have been undertaken to probe into parameters that affect cutaneous irritation. Although extensive information is available on irritant dermatitis, little is known about the pathophysiology and complexity of the underlying events. Recently, various studies with SLS as an experimental model for skin irritation have been conducted to study age and regional variability (Van der valk, 1989; Cua et al., 1990), racial differences (Berardesca and Maibach, 1988a,b), stratum corneum (SC) turnover (Wilhelm et al., 1990), sexual differences (Lammintausta et al., 1987) and the cumulative effect of irritants with repeated patch tests (Freeman and Maibach, 1988; Lee and Maibach, 1993).

Surfactants alter skin barrier function and morphology as characterized by erythema, dryness and scaling of epidermis (Imokawa et al., 1975; Agner and Serup, 1989). These reversible events could be the result of interaction between

* Corresponding author.

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surfactants and α-helical keratin proteins. Denaturation of these proteins due to conformational alterations may possibly be implicated in increased water absorption leading to surfactant induced swelling of SC (Putterman et al., 1977; Faucher and Goddard, 1978; Blake et al., 1986; Rhein et al., 1986). The degree of hydration of epidermis in vivo is correlated to the irritation potential of the anionic surfactants (Berardesca et al., 1990). The water content of SC in vivo can be quantitated by non-invasive techniques (Agner and Serup, 1990). The measurement of transepidermal water loss (TEWL) is routinely used to evaluate cutaneous irritation and integrity of SC (Wilhelm et al., 1989; Wilhelm and Maibach, 1990; Van Neste and Brouwer, 1992). The use of skin capacitance to study hydration dynamics of SC has also been reported (Wilhelm et al., 1993).

Malten (1981), suggested that the irritants can provoke an 'orthoergic (= irritant) reaction' in which the dermatitis is only a 'tip of an iceberg' of the functional alteration in epidermis. Repeated patch tests with SLS produce an augmented irritation response, indicating that healed contact dermatitis can flare again after exposure to mild irritation (Freeman and Maibach, 1988). A cumulative irritation effect of SLS using repeated open application on subclinically irritated skin has also been investigated (Lee and Maibach, 1993).

Malten and Thiele (1973) proposed that partial exposure of the skin to the same or different detergent, which was preexposed to certain detergents, may influence the intensity of irritation reaction. Following such treatment a 'crescent effect' was reported, wherein the partial overlapping of preexposed skin exhibited a milder reaction compared to adjacent, newly exposed skin. No further work has been offered to verify the occurence of this phenomenon. The present study was undertaken to investigate this crescent effect using a single surfactant SLS as the model irritant. Three distinct areas, viz. preexposed, reexposed and newly exposed, were created on a single field to simulate this effect. Visual scoring, TEWL and capacitance readings were obtained to demonstrate cumulative irritancy and potential hydration effects in these zones.

2. Materials and methods

2.1. Subjects

Patches were applied on 11 healthy subjects between 20 and 50 years of age after they provided informed consent. They were free of skin disease and had no history of atopic dermatitis. During the course of study, topical application of moisturizers or any other cream was prohibited. Patch tests were placed on the same site in all volunteers to avoid regional variation effects (Cua et al., 1990).

2.2. Materials

Sodium lauryl sulfate (99% purity, SLS: Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in deionized water at 1% concentration solution. Deionized water was used as vehicle for the control.

Polypropylene chamber patch tests with nonwoven cotton pads of 20 mm diameter (Hill Top Lab. Cincinnati, OH, U.S.A.) were used. 20 mm diameter chambers were chosen to achieve three distinct areas (preexposed, partially overlapped and newly exposed adjacent area) on a single field and thus obtain a more reproducible effect. The occlusive chambers were fixed to the skin with a nonocclusive paper tape (Scanpor; Norgesplaster A/S, Oslo, Norway).

2.3. Method

Polypropylene occlusive patches containing 1% SLS in aqueous solution were applied for 24 h on the volar side of each forearm. The first patch was applied on Monday and removed after 24 h; parameters, viz. visual scoring, TEWL, and capacitance, were recorded daily for the whole week until Friday. The second patch was then applied as described below on the following Monday, i.e., 8 days after the first patch was applied, and the experimental protocol was repeated for the following week as discussed earlier on different treated sites.

200 μl of freshly prepared SLS solution was pipetted onto the test patch just prior to applica-

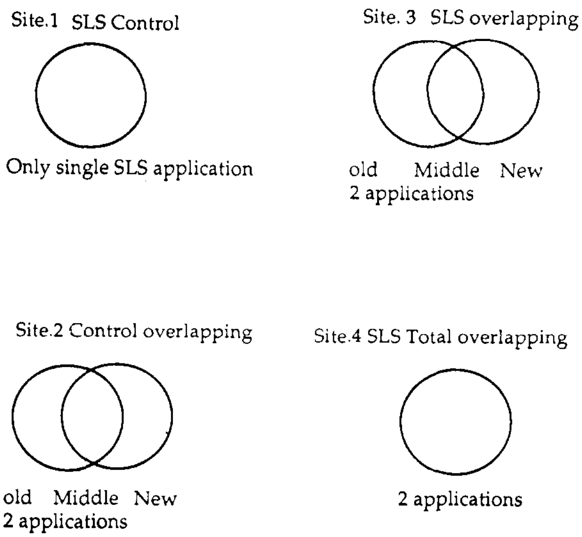

Fig. 1. Schematic representation of experimental strategies: (old) preexposed area, first application, day 1; (Middle) overlapping area, second application, day 8; (New) adjacent area, first appliaction, day 8.

tion to the skin, to prevent drying of filters. Four sites were chosen on the forearms of each subject – two patches were applied on each forearm as described below (also refer Fig. 1).

(1) SLS – first patch – day 1 – single patch (scheme 1).

(2) Deionized water – first patch – day 1 and repeated application with partial overlapping on the preexposed area on day 8. This site served as control (scheme 2).

(3) SLS – first patch – day 1 and repeated application of SLS with partial overlapping site on preexposed area on day 8 (scheme 2).

(4) SLS – first patch – day 1 and repeated application of SLS with total overlapping on the preexposed site on day 8 (scheme 3).

The chambers were removed 24 h after each application, the sites marked and wiped with a soft paper towel to remove any residual solution, rinsed with distilled water and gently dried with a soft paper towel. The volunteers were allowed to rest for at least 1h with the test sites exposed to room temperature so as to resolve tape reactions and allow excess free water to evaporate.

2.4. Visual scoring

Visual scoring was evaluated using the following scale: (1) slight erythema, either spotty or diffused; (2) moderate erythema; (3) intense erythema with papule formation.

2.5. TEWL measurements

TEWL values were recorded with an evaporimeter (Evaporimeter EPI; Servomed, Vallingby, Sweden). The method for evaporimetry using the difference in water vapor pressure gradient on the surface of the skin has been discussed elsewhere in detail (Nilsson, 1977). The probe was held in position for sufficient time (30–60 s) before recording the TEWL values to attain a stable value at each site. The relative air humidity and room temperature were uncontrolled but the values were recorded (varied between 52 and 65% RH; temperature, 18–22°C) daily. Relative humidity fluctuations were small compared to the variability measurements for detection of a correlation. The surface skin temperature was measured using a thermistor (Tele-Thermistor, Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). TEWL values were corrected to a standard skin reference temperature of 30°C as described by Mathias et al. (1981). The values are expressed as g/m^2 per h.

2.6. Capacitance measurements

The corneometer (CM 820, Courage & Khazaka, Cologne, Germany) was used to register the electrical capacitance of the skin surface (Blichmann and Serup, 1988). The capacitance is expressed digitally in arbitrary units (a.u). The principle of this instrument is to decipher distinctly different dielectric constants of water (approx. 81) and other constitutional materials (less than 7), with a probe applied to the skin, at constant pressure.

2.7. Statistical evaluation

Differences in TEWL and capacitance measurements between control and SLS treated sites were tested for significance using Student's *t*-test for paired samples.

3. Results and discussion

All subjects reacted visibly to the first exposure of the SLS patch test with mild to moderate erythema, scaling and slight infiltration on the treated sites. The control area exhibited no such reaction, except for mild erythema in two subjects. On second exposure, the overlapping area (both partial and total) displayed moderate to severe erythema with papule formation in three subjects. The area adjacent to the partially overlapped site which was exposed for the first time to SLS on day 8 also showed moderate to severe erythema.

3.1. TEWL

After the single 24 h patch application of 1% SLS, the TEWL measurements were approx. 5-times higher than the normal baseline value. A gradual decline was then observed and near constant values were attained from day 8 post patch removal. However, the return to normal baseline value was not achieved 12 days post treatment (Fig. 2), suggesting either slow recovery or permanent damage to skin following prolonged treatment with SLS.

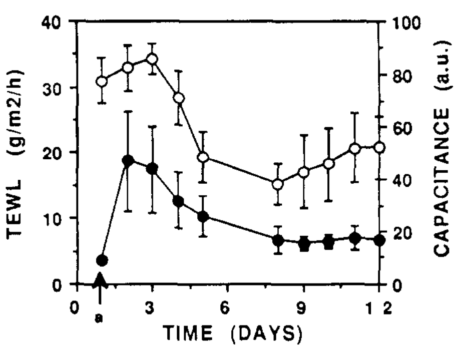

Fig. 2. TEWL and capacitance measurements after 24 h application of 1% SLS to the volar forearm of human volunteers. Values are represented as mean ± SD ($n = 11$). (●) TEWL; (○) capacitance; a, first application.

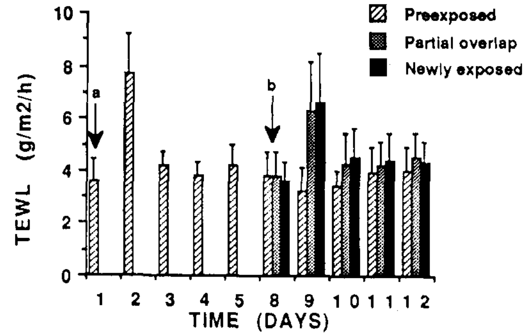

Fig. 3. TEWL measurements after 24 h application of water (control) to the volar forearm of human volunteers. Values are represented as mean ± SD ($n = 11$). a, first application; b, second application – partial overlap.

Using water as the control (in the absence of SLS), a 2-fold increase in TEWL was observed which dropped to normal baseline values on day 3. The normal values were maintained over the 12 day study period. Reapplication of water on day 8 (scheme 2) augmented TEWL in partially overlapped (PO) and newly exposed (NE) areas (Fig. 3). Consistent with the first application, baseline values were again established for PO and NE sites after 24 h post patch removal. Comparison of PO and NE sites, however, revealed no statistical difference in TEWL values. The above results and comparable area under the curves for preexposed, PO and NE areas suggest a simple, short and uniform hydration effect of water on skin.

The reapplication of SLS on day 8 according to scheme 2 elevated TEWL values in both PO and NE areas (Fig. 4). A slightly higher response was observed in the PO area on day 9 probably due to the cumulative effect of SLS as reported in other studies (Freeman and Maibach, 1988; Lee and Maibach, 1993). This was further confirmed when comparable TEWL values and area under the curves (AUC) were obtained for the PO area according to scheme 2 and also for the TO site according to scheme 3. The AUC for the PO area (scheme 2) lies between the AUC after single SLS treatment (scheme 1) and that after repeated application of SLS, at the same site (scheme 3). The above findings suggest possible radial migration of SLS away from its site of

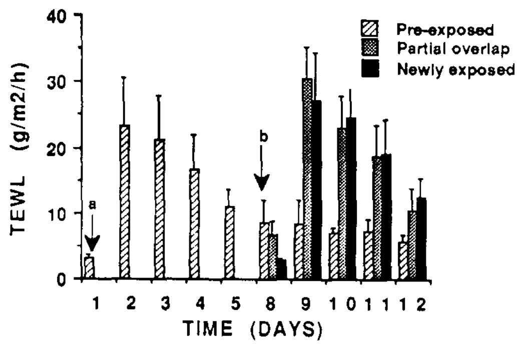

Fig. 4. TEWL measurements after 24 h application of 1% SLS to the volar forearm of human volunteers. Values are represented as mean ± SD ($n = 11$). a, first application; b, second application – partial overlap.

application. Indeed, lateral spread of compounds has been demonstrated (Guy and Maibach, 1982; Albery et al., 1983). SLS as an irritant, on prolonged exposure can induce vasodilation (Van Neste et al., 1986) and thus encourage self radial spread. The obvious implication of this finding is the possible susceptibility of an area to irritation not directly exposed to SLS. Further work is, however, needed to quantitate the radial diffusion of SLS away from its site of application.

The prolonged use of SLS is associated with formation of dry and scaly epidermis due to dehydration, resulting from excessive loss of bound water from superficial skin (Agner and Serup, 1989) and free water from underlying tissues. Accordingly, the TEWL measurements for PO and NE areas (scheme 2) declined from day 10 onwards. However, TEWL measurements on days 10–12 were always higher for NE sites relative to the PO area in seven out of 11 subjects ($P < 0.03$). The repeated use of SLS may be associated with more dehydration in the overlapping areas thus causing a rapid decline in TEWL as compared to freshly exposed and relatively more hydrated area. The TO sites also displayed an augmented irritant effect (Fig. 6) as reported in previous studies. These values decreased subsequently, yet remained elevated even on day 12 compared to controls.

The inability of TEWL values to return to pretreatment values following SLS treatment (in contrast to control) suggests alterations in skin barrier properties which cannot be entirely attributed to hydration effects. The relative contributions of specific water induced and SLS induced alterations in skin barrier properties were estimated by calculating the AUCs of TEWL measurement vs time plots for water and SLS treatments, respectively. Assuming a damage factor of unity, the fractional contributions of specific SLS and water induced skin alterations were observed to be 0.57 and 0.43, respectively. The relatively higher contribution of SLS highlights the individual effects of SLS on skin independent of water induced skin hydration. One interpretation of the above observation may be the increased skin hydration caused by SLS by either (1) interacting with hydrophobic groups of the membrane, thus creating new water binding sites (Rhein et al., 1986) or (2) uncoiling the protein helical rings resulting in a net increase in the effective surface area for water absorption. Another possible interpretation is the fluidization of skin lipids by prolonged SLS treatment (Rhein et al., 1986).

3.2. Capacitance measurements

The capacitance studies give an estimate of the hydration capacity of the skin (Mosler, 1983; Wilhelm et al., 1993). The degree of healing and hydration level of the epidermis were correlated with the recovery of skin capacitance values. Following single 24 h application of 1% SLS, capacitance values increased slightly over 3 days and then declined over 4–8 days followed by a slow but incomplete recovery over 8–12 days (Fig. 2). The initial increase in capacitance is probably due to increased skin hydration induced by SLS and the subsequent decline once more suggests a possible dehydrating property of SLS on prolonged application to skin. Consistent with TEWL measurements, capacitance values also did not return to pretreatment values even after 12 days, again indicating slow recovery or permanent damage to the skin. The inability of both TEWL and capacitance measurements to return to pretreatment levels after 12 days following 24 h exposure to SLS strongly suggests the detrimental effects of SLS on skin lipids in addition to its well known hydration effects. Surfactants are also known to remove the intercellular lipids present in the intercorneocyte domains (Rhein et al., 1986). Consistent with our results, Elias (1981) has reported that the removal of these SC lipids lead to an increase in transcutaneous water loss. Another study (Abrams et al., 1993) using lipid solvent systems suggested that the increase in TEWL is possibly due to solvent-protein binding that displaces the water from epidermal keratin proteins. Since surfactants are known to affect both lipids and keratin proteins, the increase in TEWL can thus be attributed to the effect of SLS on these components of the corneocyte layer. The dehydration of SC after 72 h of patch removal may therefore be due to excessive loss of water which eventually leads to an altered skin barrier function.

The reapplication of SLS on day 8 at the PO test site resulted in elevation of capacitance values in both PO and NE areas (Fig. 5). A stronger response was noted for the PO area as compared to the NE area which is consistent with TEWL studies and may again be attributed to the cumulative effect observed for the PO area. This cumulative effect was again evident when SLS was reapplied at the TO site (Fig. 6), whereby reapplication of SLS on the same site on day 8 produced significantly higher capacitance values. However, the capacitance levels declined thereafter from day 10, suggesting dehydration of SLS

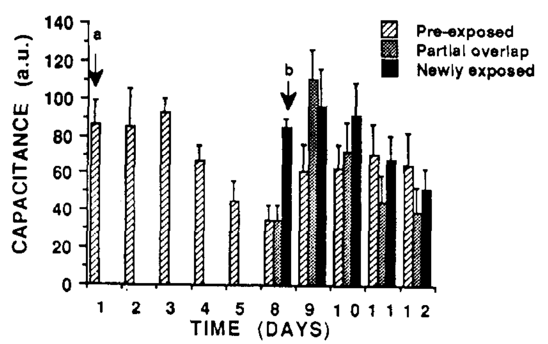

Fig. 5. Capacitance measurements after 24 h application of SLS to the volar forearm of human volunteers. Values are represented as mean ± SD ($n = 11$). a, first application; b, second application – partial overlap.

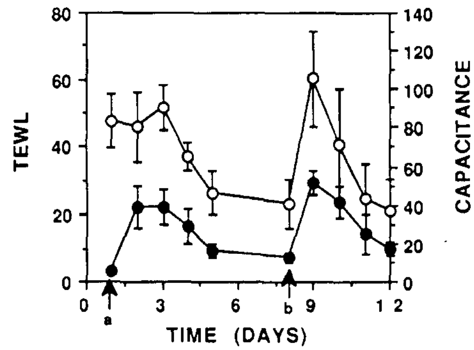

Fig. 6. TEWL and capacitance measurements after 24 h application of 1% SLS to the volar forearm of volunteers. Values are represented as mean ± SD ($n = 11$). a, first application; b, second application – total overlap.

treated skin. No significant changes in skin capacitance were observed when water alone was used as control, which further highlights the role of SLS relative to water in altering barrier properties of the stratum corneum. As evident with TEWL measurements, the capacitance levels for the PO area on days 10–12 were always lower compared to the NE area ($P < 0.01$), implying that reapplication of SLS at the same site causes excessive loss of bound water compared to freshly exposed area. The reduction in water absorbing/binding properties of SC on repeated exposure is probably due to denaturation of proteins (Rhein et al., 1986).

It has been suggested that absorbed SLS binds to keratin and induces swelling (Putterman et al., 1977; Faucher and Goddard, 1978; Blake et al., 1986). Moreover, mechanisms responsible for hydration are further related to irritation properties (Rhein et al., 1986). Although TEWL values approach near normal level by 1 week after the treatment, skin capacitance commences recovery only 1 week after SLS exposure. These results suggest that although the epidermis shows signs of recovery from damage over 1 week after surfactant treatment, the real damage caused is much extended. Actual healing as evident with our hydration measurements suggests that a longer time is needed for attaining the normal hydration of SC after SLS treatment than what was believed to be the case. Moreover, the capacitance data instigated us to propose that TEWL readings alone do not portray the degree of damage to skin with irritants. Recording the capacitance values over a period of time may in turn give a better insight into the actual time taken for the skin to attain normal hydration state after surfactant treatment.

Is cumulative irritancy a constant phenomenon in general? Our present results agree with the earlier findings wherein an augmented effect was seen with repeated exposures to irritants, although a large degree of interindividual variation was evident with partially overlapped sites. Although we observed the crescent effect using one surfactant, as suggested by Malten and Thiele (1973), we believe that this overlapping region, in fact, exhibits an intense irritation effect characteristic of cumulative irrtation and the higher TEWL values seen in the NE area are probably due to the reasons explained above. The possibility of occurrence of this phenomenon as suggested by Malten and Thiele using two different surfactants still needs to be elucidated and is currently being investigated in our laboratory.

The implications of this study are:

(1) Repeated application of SLS may cause excessive dryness due to the cumulative dehydrating effects of SLS when compared to freshly exposed areas.

(2) Prolonged SLS treatment (1%, 24 h) damages the skin barrier in such a way that it either takes considerable time to recover or possibly leads to prolonged subclinical damage.

(3) TEWL and capacitance measurements are good independent (and complementary as from our studies) tools to quantitate stratum corneum hydration in vivo.

(4) Although speculative, radial spread of SLS may occur following prolonged treatment which may sensitize the skin adjacent to the treated site.

(5) The mechanism of SLS action may be either (1) by causing increased hydration independent of the water effect (such as binding to keratin) or alternatively by fluidizing/extracting the skin lipids on prolonged exposure.

(6) The inability of the TEWL and capacitance measurements to return to baseline levels following prolonged exposure to SLS strongly suggests interaction of SLS with lipids in addition to hydration effects.

(7) The reduction in water binding properties of the stratum corneum on repeated exposure suggests denaturation of proteins.

Taken together, the surfactant effects on skin are complex and when viewed at a subclinical (bioengineering) level, offer some insights that may help unravel the equally complex issues of clinical irritant dermatitis in man.

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