

Review Article

Irritants in combination with a synergistic or additive effect on the skin response: an overview of tandem irritation studies

FRANCISCA KARTONO¹ AND H. I. MAIBACH²

¹Department of Dermatology, UCSF, School of Medicine, San Francisco, CA, USA and ²Western University of Health Sciences, Osteopathic School of Medicine, Pomona, CA

Sodium lauryl sulfate (SLS) has often been chosen as a model for irritant contact dermatitis (ICD) to study the effect of irritants in combination (1–14). Recently ‘tandem’, or sequential, exposures with SLS have been performed to study the mechanism of skin barrier impairment in ICD (1–6, 15). The assessment of reactions have been documented with visual scoring, transepidermal water loss (TEWL), skin colour reflectance measurements, skin blood flow; among which TEWL has been noted as the most sensitive value (16). The matched control groups were treated with either a single exposure to a single irritant or in tandem with the same irritant repeatedly. Synergistic and additive effects have been reported for various tandem pairs of irritants, however, the mechanism for both remains unclear. The results of tandem irritation studies were evaluated to define and investigate the responses produced and deduce a possible mechanism of action. Clinical ramifications, albeit complex, are discussed.

Key words: combined exposures, irritant contact dermatitis, skin permeability, synergy, tandem irritation. © 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard.

Accepted for publication 01 December 2005

Occupational skin disease is an important health burden in society and causes a significant impairment in the quality of life of employees (17); where the majority of the reported cases suffer from irritant contact dermatitis (ICD) of the hands (18). ICD is a non-immunological local inflammatory reaction characterized by erythema, oedema, or corrosion following single or repeated application of a chemical substance to an identical cutaneous site (19). Studies have investigated and quantified the effects of single irritant exposures, in particular sodium lauryl sulfate (SLS) which has been studied as a model irritant (7–14). However, irritation dermatitis in the workplace generally occurs with a combination of exposures to multiple irritants rather than with just a single irritant. Studies on ICD with multiple irritants have been studied recently to simulate conditions in the workplace and in consumers in a calculated and valid methodology. Various experimental designs have been used by research groups to analyse the interaction between multiple irritants in ICD, making it difficult to interpret and compare the data across studies in order to draw a conclusion (1–14).

Recent studies have shown through ‘tandem’ or sequential applications of different agents that the cutaneous response in ICD can be different compared to repeated exposures to a single irritant. The coined term Tandem Repeated Irritation Test (TRIT) has been reported as having potential in the study of repeated exposure to multiple irritants in contact dermatitis (15). The results observed in TRIT studies have shown various modification of the cutaneous response compared to the response seen with exposure to single irritants. Depending on the pairs of irritants investigated, several types of cumulative irritation may result: (i) additive, a tandem response which equals the sum of the expected responses from each irritant alone; (ii) synergistic, a tandem response which is far greater than the sum of the expected response from each irritant alone; (iii) quenching, a tandem response much less than the sum of the expected response to each irritant alone. We review the development of tandem irritation studies and the possible mechanisms that may lead to the responses seen with such irritation studies. Knowledge of the trend in tandem irritation response can guide us

in selecting subsequent pairs of irritants to use in future studies to elucidate the mechanism of action behind skin permeability barrier disruption in ICD.

Measurements

Researchers have followed disparate procedures when conducting tandem irritation studies in the past, thus making comparisons across studies difficult. The most recent tandem irritation studies however, have been performed using similar study designs (3–6). Studies were performed in single-blinded, randomized manner, under standard laboratory conditions at room temperature 20–22 °C and 30–40% relative humidity. Subjects underwent acclimatization periods of 15–30 min. Application areas are on the paravertebral midback or volar forearm, with 12 mm Finn Chambers (Epitest, Helsinki, Finland) under occlusion. The first irritant was applied on the filter discs, and removed after 30 min. The area was carefully rinsed with 10 ml tap water and blotted dry with paper tissue. After 3 h, the

second irritant exposure was performed. This procedure was repeated for 4 days in each case at the same time of the day ± 1 h.

Assessment of the irritant potential of chemicals in ICD has been investigated through various methods: visual scoring, transepidermal water loss (TEWL), laser Doppler flowmetry, electrical capacitance and skin colour reflectance. Visual scoring has been performed in different ways. Several studies utilized the scale of Frosch & Kligman (20), based on three main types of skin lesions: erythema, scaling, and fissuring (erythema: 1 + slight redness (spotty or diffuse), 2 + moderate and uniform redness, 3 + intense redness, 4 + fiery redness; scaling: 1 + fine, 2 + moderate, 3 + severe with large flakes; fissures: 1 + fine cracks, 2 + single or multiple broader fissures, 3 + wide cracks with haemorrhage or weeping). Whenever the visual score developed to a value ≥ 5 in a single test field (cut-off criterion) the exposure to the irritant was prematurely discontinued. Other studies employed the scale of Willis et al. (21), using the erythema scale alone as their visual scoring criteria, with similar cut-off criterion.

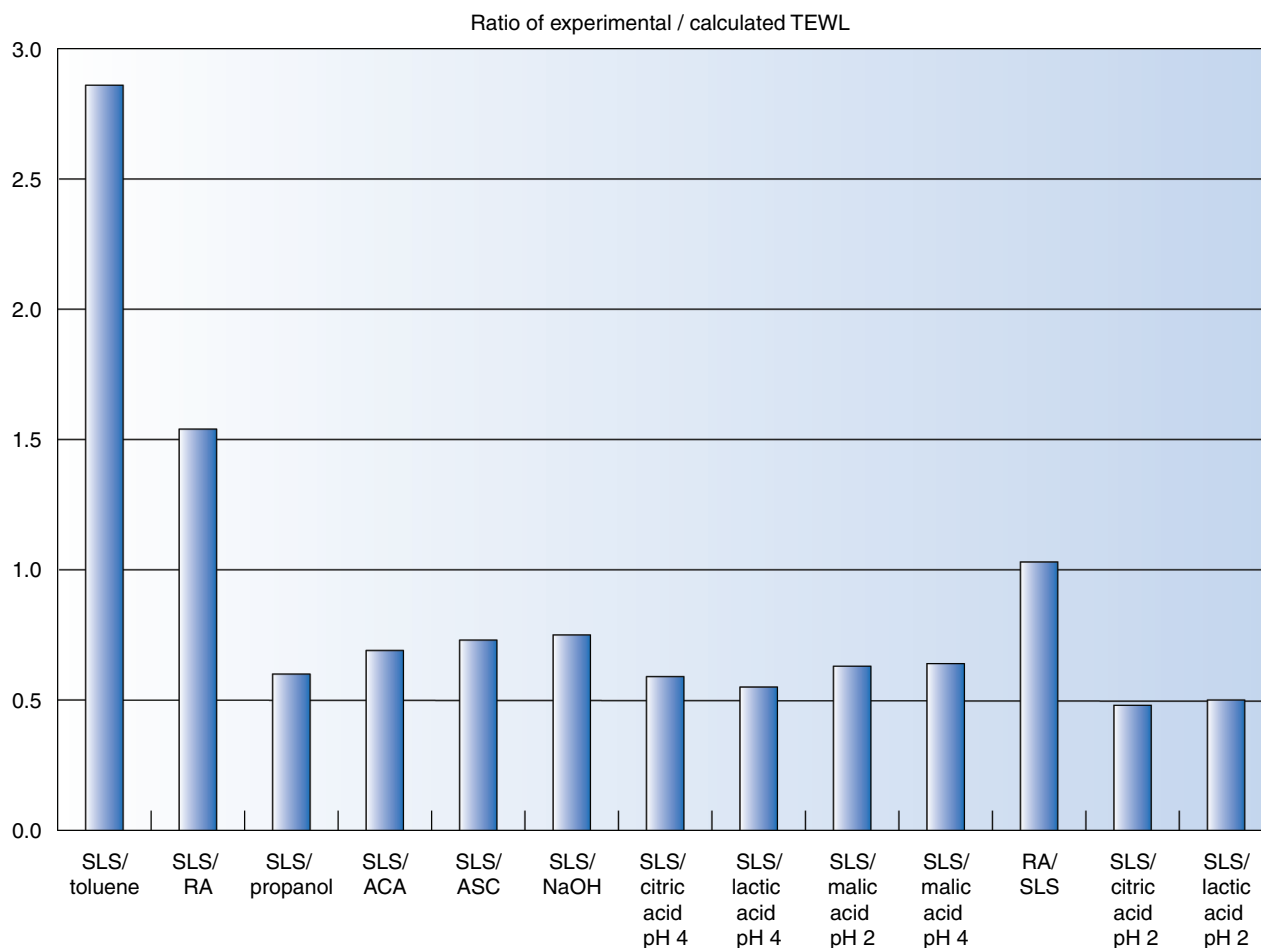


Fig. 1. Comparison of ratio of experimental/calculated transepidermal water loss.

Transepidermal water loss, expressed in $\text{g/m}^2/\text{h}$, is an indicator of the water vapour pressure between the boundary layer of the skin surface and the ambient air. It reflects the integrity of the epidermal permeability function under normal or barrier-perturbed conditions such as in compromised or diseased skin. An evaporation meter is used in most of the recent tandem studies to measure TEWL in accordance with the Guidelines of the Standardization Group of the European Society of Contact Dermatitis (ESCD) (22). TEWL has been reported as the most reliable measurement in assessing skin barrier impairment compared to other bioengineering methods (16). Gender, race, and age does not have a cause a significant difference on TEWL, with skin of forearm and back having similar results for TEWL. However, interrater and intrarater difference may exist between opposite arms, and before and after exposure (22).

Laser Doppler flowmeter measures cutaneous blood flow. The Doppler-shifted light is back-scattered from moving red blood cells, while unshifted laser light is back-scattered from stationary tissues. The blood flow signal was expressed in relative and dimensionless values (23).

Electrical capacitance indicates the hydration level of the skin and can be measured using a corneometer CM 825 (24).

Measurements of skin colour were conducted using a chromameter with the guidelines from Elsner (25) or other published guidelines (26, 27). The instrument measures colour reflectance and computes the chromatic dimensions of colour by means of the L^*a^*b 3-d dimensional colorimetric system. To quantify erythema, the a^* value is of specific interest in measuring the red (positive value) and green (negative value) distinction.

Mechanical Irritants

Conditions that may support the induction of barrier disruption and subsequent ICD include mechanical and chemical irritation. We can distinguish the tandem irritation studies between mechanical and chemical irritation. Mechanical irritants in the workplace have been reported in the form of influences from air conditioning, climatic changes, temperature changes, airflow (28, 29). Chemical irritants on the other hand, come in a vast array from detergents and perfume exposures in housewives to that of fruit acids in bakers (7, 30, 31).

Temperature

Interactions between mechanical and chemical irritants have been studied since 1977 when

Rothenborg et al. described temperature dependent irritant dermatitis from lemon perfume (7). In a study from an outbreak in cleaning personnel from using a lemon scented detergent, it was shown that exposure to 43 °C of the lemon perfume component citral proved to be a primary irritant (toxic) dermatitis even at 48 h after the exposure. This was in contrast to the reversible skin reactions seen with citral at 23° which had reduced by 3 h after the exposure. A similar experiment to observe effects of low temperature was done by Halkier-Sorensen et al. (32) Findings reported a reduction in erythema, itching, but no apparent effects on local oedema.

These findings led to subsequent experiments to assess temperature dependent skin damage. Berardesca et al. reported the effect of increasing temperature on surfactant (5% SLS) induced irritation on the skin. Indeed damage was progressive according to increasing temperature (9). A supporting finding by Øhlenschläger showed that by immersions of the forearm into 40 °C 0.5% SLS, significant TEWL occurs as compared to at 20 °C SLS. However, SLS-only immersion experiments showed a reduction in skin capacitance regardless of temperature at 40 or 20 °C, even though TEWL did not show significant changes (10). A subsequent study proposed that the anionic character of the detergent and temperature have a positive correlation to the irritant potential (11). The study compared irritant effects by SLS with a milder detergent where 50% of the anionic molecules were replaced by non-ionic molecules (lauryl glucoside). The solutions were then tested at 37 and 40 °C, and compared to each other; the increase in temperature and anionic detergent content indeed caused a stronger response.

In contrast to the many studies conducted on the effect of an increase in temperature, few studies were reported to evaluate the effect of cold in relation to detergent irritation. A study on the relevance of low skin temperature in fish processing employees showed that coldness induced on the skin by ice cubes to 15 °C significantly reduce the symptoms seen with histamine scratch testing otherwise pronounced at room temperature (32). A reduction was seen in itching, erythema, and skin blood flow. However the extent of the wheal reaction stayed constant, meaning capillary permeability was not much affected by cooling. Fluhr studied the effects of 15 and 4 °C of 0.5% and 1% SLS and its effects on TEWL, visual scoring and skin colour reflectance (12). Indeed coldness at both temperatures decreased TEWL and visual scoring, however, a decrease in redness by skin colour reflectance was not observed.

Table 1. Mechanical irritation studies simultaneously and in tandem

Design	Irritant/vehicle	Materials and methods	Result	Reference
Simultaneous heat + irritant	Citral 10% in alcohol	Ventral forearm with Finn chambers. 20min under occlusion. Evaluated by biopsy.	Strong toxic acute dermatitis only seen at high temperature (43 °C).	(7)
Simultaneous heat + irritant	SLS in water	In vitro skin. 2 h immersion then at 75°–90° in oven by DSC. Evaluated by DSC, TEWL, XRD.	Increased temperature causes disorganized lipid pattern on XRD. SLS decreases lipid Tm.	(8)
Simultaneous heat + irritant	SLS in water	Ventral forearm. Open application then air dried. Evaluated by TEWL, erythema, colorimetry, capacitance.	Increased TEWL, erythema colorimetry, and decreased capacitance seen at 20°, 40°, but not at 4°.	(9)
Simultaneous heat + irritant	SLS in water	Arm immersion at 20° and 40°. Evaluated by TEWL, capacitance, erythema.	SLS reduces capacitance at 20° and 40°. Increased TEWL at 40°, not at 20°.	(10)
Simultaneous heat + irritant	SLS (anionic) in water	Arm immersion at 37° and 40°. Evaluated by TEWL, capacitance and erythema.	More increase in TEWL, erythema and less of a decrease in capacitance at 40° than 37°.	(11)
Simultaneous cold + irritant	Histamine 0.3%	Ventral forearm scratch test at room temperature and after ice cube application. Evaluate for erythema, SBF, wheal.	Cold reduces erythema by 50%, reduces itch, but does not reduce wheal formation.	(32)
Tandem cold + irritant	SLS in water	Paravertebral midback with Finn chambers at 4° and 15°. Evaluated by VS, TEWL, colorimetry.	SLS/cold and cold/SLS decreases TEWL. No changes in other measurements.	(12)
Tandem airflow + heat + irritant	SLS in water	Paravertebral back with Finn chambers. Evaluated by VS, TEWL, colorimetry.	Additive effect in airflow/SLS and SLS/airflow.	(13)
Tandem mechanical irritation + irritant + occlusion	SLS in water	Ventral forearm with Finn chambers. Evaluated by VS, TEWL, colorimetry.	Occlusion (by water, glove or SLS)/mechanical irritation caused more increase in TEWL than mechanical irritation/occlusion. Longer period of occlusion increases TEWL.	(14)

SLS, sodium lauryl sulfate; DSC, differential scanning calorimetry; TEWL, transepidermal water loss. Temperatures are reported in °C.

Table 2. Chemical irritation studies simultaneously and in tandem

Design	Irritant/vehicle	Materials and methods	Result	Reference
Tandem chemical irritants	RA in ethanol. SLS in water	Ventral Forearm with occlusive polypropylene chambers. Evaluated by TEWL, VS, SBF, capacitance.	Synergistic effect in SLS/ RA Additive effect in RA/SLS	(1)
Tandem chemical irritants	RA in ethanol. SLS in water	Paravertebral midback with Finn chambers. Tandem interval time was varied. Evaluated by VS, TEWL, colorimetry.	Increased reaction at day 1–2, remains same at day 7, resolves at day 7–14 after the injury.	(2)
Tandem chemical irritants	SLS in water Toluene, undiluted	Forearm with Finn chambers. Evaluated by VS, TEWL, colorimetry.	Synergy seen with SLS/toluene and toluene/SLS.	(3)
Tandem chemical irritants	SLS in water n-propanol in water	Ventral forearm with Finn chambers. Evaluated by VS, TEWL, colorimetry.	Limited additive effect seen with SLS/n-propanol	(4)
Tandem chemical irritants	Ascorbic acid in water Acetic acid in water NaOH in water SLS in water	Paravertebral midback with Finn chambers. Evaluated by VS, TEWL, colorimetry.	Additive effect in TEWL for ASC/SLS, ACA/SLS, NaOH/SLS.	(5)
Tandem chemical irritants	Citric acid in water Lactic acid in water Malic acid in water SLS in water	Paravertebral midback with Finn chambers, at pH 2 and pH 4. Evaluated by VS, TEWL, colorimetry.	Quenching effect with lactic acid/SLS and citric acid/SLS at pH 2. Additive effects with other combination tandem studies.	(6)

SLS, sodium lauryl sulfate; DSC, differential scanning calorimetry; TEWL, transepidermal water loss; XRD, X-ray diffraction; VS, visual scoring; SBF, skin blood flow; RA, retinoic acid; ASC, ascorbic acid; ACA, acetic acid. Temperatures are reported in °C.

This apparent protective effect of coldness on skin barrier impairment, however, is contradicted in another study by Halkier-Sorensen and a disruption in homeostasis is actually suggested as an influence of coldness (33).

However, the previously mentioned studies on the effects of temperature on skin barrier were not performed as tandem irritation studies, but rather in a combined fashion. Therefore we could not evaluate for synergistic or additive interaction of temperature and SLS. Heated or cooled SLS was prepared at different temperatures and tested on the skin surface. Øhlenslæger et al. showed 0.5% SLS immersion of the forearm at 20 and 40 °C, and then compared to water immersion at the same temperatures. A significant increase in TEWL was only observed as a combined effect of SLS and increased temperature and not when SLS or temperature increase was introduced alone. This can be explained as increased temperature causes an increase in water flux through the stratum corneum (SC). Increase in water flux can increase penetration of SLS and subsequently increase the impairment of barrier function.

Airflow

The last 2 studies published by Fluhr on mechanical irritation involved testing the effects of

airflow and temperature in tandem with SLS (13). Paravertebral back skin areas were exposed to airflow at different velocities at 24 and 43 °C in combination with 0.5% SLS in a tandem repeat fashion. Temperature or airflow alone did not have a significant effect on TEWL in the tandem studies. It was also concluded that both velocities (60 and 90 m³/h) did not have a statistically significant effect on TEWL, although the authors noted that the slower airflow induced irritancy more rapidly. They concluded that exposure to airflow in tandem with SLS has an additive effect in skin barrier disruption.

Mechanical irritation

A recent study investigated the effect of mechanical irritation with a brushing machine in tandem with water occlusion and occlusion with 0.5% SLS (14). Irritation was then assessed by visual scoring, skin colour reflectance, and TEWL. They concluded that mechanical irritation alone caused a mild, but significant increase of TEWL values on the last day of the experiment. Furthermore, occlusion followed by mechanical irritation induced a stronger barrier function impairment than mechanical irritation followed by occlusion. The study also showed that increase in length of occlusion time (by water, glove, or SLS) caused an increase in TEWL. This finding

Table 3. Additive, synergistic, and quenching results of tandem studies (1–6)

Interaction*	Tandem irritants	Ratio of experimental/ calculated TEWL†
Additive	SLS/propanol	0.60
	SLS/ACA	0.69
	SLS/ASC	0.73
	SLS/NaOH	0.75
	SLS/citric acid pH 4	0.59
	SLS/lact c acid pH 4	0.55
	SLS/malic acid pH 2 and pH 4	0.63
	RA/SLS	1.03‡
Possible synergy	SLS/Toluene	2.86
	SLS/RA	1.54‡
Possible quenching	SLS/citric acid pH 2	0.48
	SLS/lactic acid pH 2	0.50

*Interaction was arbitrarily defined by ratio of experimental/calculated TEWL values: (i) additive: $0.51 \leq \text{ratio} \leq 1.50$, (ii) synergistic, ratio ≥ 1.50 , (iii) quenching: ratio ≤ 0.50 . Day 5 TEWL values were used for ratio calculation, baseline values at day 0, when provided in the paper, were subtracted from the TEWL values.

†Calculated TEWL is obtained by taking the average of TEWL resulting from repeated tandem exposure to each of the irritants alone as TEWL from single exposure to each of the irritants were not provided in the papers. The ratio is then obtained by dividing the measured TEWL by the calculated TEWL.

‡In case of RA, the calculated TEWL was obtained using the sum of TEWL resulting for single exposure to each of the irritants alone. The ratio is then obtained by dividing the measured TEWL by the calculated TEWL. Day 3 TEWL values were used for ratio calculation, baseline values at day 0, when provided in the paper, were subtracted from the TEWL values.

was attributed to an initial dissolution of SC integrity, especially by SLS (34).

Chemical Irritants

Numerous studies on the effect of chemical irritants have used SLS as a model irritant in comparison to the more recently studied chemical irritants. A well accepted dose dependent response of SLS irritation has been reported. The mechanism behind disruption of skin barrier by detergents, especially SLS, has been thought to cause a decrease of lipid melting point and an increase in water diffusion (8). Ribaud et al. investigated the effects of SLS on the barrier function of *in vitro* skin tissue. SLS introduced lipid changes similar to heat treatments, which causes a decrease in lipid melting temperature from the critical micellar concentration of SLS (0.24%) (8). A decrease in the intensity of diffraction pattern peaks with increasing SLS concentration was also observed. Both changes indicate a disorder pattern that develops in the intercellular lipid organization with the application of SLS on human skin. The mechanism of this permeability alteration remains *sub judice*.

Barrier function of the SC is mainly due to ceramides, however, studies by Froebe et al. indicated that ceramides were not extracted by SLS (35). When applied topically, SLS is an acute irritant, by a direct cytotoxic effect on keratinocytes, affecting both lipid and protein structures. SLS can induce damage of skin barrier function, exceeding 10 days after a single occlusive application (36–37).

The subsequent tandem studies performed have used SLS as a model irritant for comparison. A study on the effect of retinoic acid (RA) on skin permeability by Effendy et al. quantified the irritant effects of 0.05% RA, 1% RA and 1% SLS on normal skin in a 24-h assay, both alone and in tandem, that was monitored for 18 days (1). Measurements were taken for visual scoring, TEWL, skin blood flow and electrical capacitance. No statistically significant difference was found between results using 0.05% and 0.1% RA, suggesting a similar potency. SLS in tandem with RA produced a significant increase in TEWL compared to RA in tandem with SLS, or compared to repeated exposure of each irritant alone. However, exposures to single irritants were not performed twice with a 24 h interval over 2 days as the tandem experiments, but the single irritant controls were performed once over a 24-h period. RA exposures also increased the amount of scaling on the skin independent of SLS application. An increase in erythema, skin blood flow, and water loss (reduced capacitance) was statistically significant with SLS followed in tandem by RA compared to RA followed in tandem by SLS. They concluded that exposure to SLS may render the SC barrier highly permeable and allow a greater penetration of the following RA application. Interestingly, analysing the data published by Effendy et al. by measuring the heights on graphs of TEWL, taking into account baseline measurements at day 0; SLS/RA tandem application caused an increase in TEWL by a factor of 1.8 of the sum of the TEWL by each irritant alone, whereas RA/SLS had an increase in TEWL by a factor of 1.03 compared to the sum on TEWL by each irritant alone. The interaction of SLS/RA in tandem seems to have a synergistic effect whereas the RA/SLS in tandem application shows an additive effect.

A following study was performed by Ale et al. on the tandem application of SLS/RA compared with SLS/ethanol, water/ethanol, and water/RA to confirm the synergism seen in the study of Effendy et al. (2) Measurements were taken for TEWL, capacitance and skin colour reflectance. The time interval between the tandem applications were varied between 1, 24, 48, 96 h, and 1

and 2 weeks. The results showed a synergistic trend in SLS/RA in all variables, except capacitance, showing an antagonistic interaction for skin hydration. It was also noted that an increased reaction occurs at 1 and 2 days, remains at 7 days and resolves 7–14 days after acute SLS injury.

The following tandem irritation study evaluated the effects of a tandem repeat study on 0.5% SLS and undiluted toluene (3). The matched controls were performed with repeated exposures to a single irritant according to the tandem application timeline, rather than as a single irritant exposure as in the previous studies with RA. The results showed a significant synergistic effect with SLS/toluene in tandem regardless of the order of application. We calculated from Wigger-Alberti et al. that TEWL was increased by a factor of 2.9 compared to the expected sum of TEWL of the individual irritants.

A subsequent study analysed the interaction in a tandem study of 0.5% SLS/60% n-propanol (4). The matched controls in this study were performed with repeated exposures to a single irritant following the tandem application timeline. Measurements were taken for visual scoring, TEWL, and skin colour reflectance. The results showed no synergistic effect and limited additive effect with SLS/propanol in tandem studies. Calculating from Kappes et al. we found that TEWL was decreased by a factor of 0.6 compared to the expected sum of TEWL of the individual irritants. It seems that a quenching mechanism rather than an additive one is at work in the interaction between SLS/propanol in tandem irritation studies.

The following study reviewed fruit acids; ascorbic and acetic acid, and NaOH in combination with SLS in tandem (5). The matched controls were again performed with repeated exposures to a single irritant according to the tandem application timeline. Measurements were taken for visual scoring, TEWL, and skin colour reflectance. The results showed that additive effects are seen in all 3 categories for SLS/ascorbic and acetic acid as well as for SLS/NaOH in tandem studies.

More fruit acid exposure were later investigated in tandem with SLS. Schlemman-Willers et al. investigated citric acid, malic acid, and lactic acid in tandem with 0.5% SLS (6). The matched controls were again performed with repeated exposures to a single irritant following the tandem application timeline. Measurements were taken for visual scoring, TEWL, and skin colour reflectance. Measuring the data published

on paper by Schlemman-Willers et al. we found that TEWL was quenched in tandem studies of lactic acid and citric acid, and additive with malic acid. More quenching was observed with the fruit acids at pH 2 rather than at pH 4. No signs of synergistic interaction was seen with any combination in the tandem irritation studies.

Discussion

The SC, the outermost layer of the epidermis, a lipid protein biphasic structure, is 10–20 μm thick in most surfaces on the human body (38) and consists of 2 compartments, a desquamating layer of protein enriched corneocytes embedded in a compact lipid intercellular matrix (39). The SC poses as the rate-limiting barrier that a compound must penetrate upon entering the skin. As a molecule finds its way into the SC, it is suggested that several pathways are available, through transcellular/intracellular diffusion, or through shunt pathways through holes or shunts left by hair follicles, glands, skin appendages (40).

Analysis of the mechanism of penetration on molecules through the SC is valuable for drug delivery research, as well as to assess toxicity to the skin after exposure to a substance. Previous approaches summarized that the free energy of solute transfer into SC lipids is a function of the size, polarity and hydrogen bonding ability of the permeant, and group contribution of the functional groups (41).

Another factor of SC penetration by solutes is the maximal delivery flux. It is the maximum dose of a solute able to be delivered over a given period of time and area of application from a given vehicle (J_{max} , $\text{mol}/\text{cm}^2/\text{h}$) (42). Magnusson et al. showed that molecular weight was a dominant determinant of J_{max} , with melting point and hydrogen bonding marginally improving the analysis (42). J_{max} for solutes with similar MW is associated with a log octanol-water partition coefficient ($\log K_{\text{ow}}$) of 2.5–3.0 and greatest for solutes with a low melting point. Among the solutes studied by tandem irritation, propanol has the smallest molecular weight (MW = 60.1), while toluene is larger (MW = 92.14), yet toluene caused a more significant and synergistic effect with SLS in TEWL. Toluene has a melting point higher than n-propanol ($T_{\text{m toluene}} = -93^\circ\text{C}$, $T_{\text{m propanol}} = -127^\circ\text{C}$), suggesting that propanol should have the greater flux if T_{m} was the determinant. Comparing the log octanol-water partition coefficient, toluene has a $\log K_{\text{ow}} = 2.70$ compared to propanol with $\log K_{\text{ow}} = 0.34$, indicating their permeation into SC to be primarily controlled

by K_{ow} . There is still a need to explore the correlation between solute flux (J_{max}) and clinical TEWL increase *in vivo*. The contribution of K_{ow} on J_{max} appears dominant in the tandem SLS/toluene experiment if a large J_{max} caused the synergistic increase in TEWL.

The role of SLS as tandem irritant

It was previously thought that solvents remove intercellular lipid material resulting in cutaneous barrier impairment. However subsequent studies show that SLS treatments alter the quality, not the quantity of SC lipids (43). SLS also play a role in lipid bilayer disorganization (8), protein denaturation, increased water uptake by SC, and swelling of the corneocytes (44, 45). The increase seen in SC hydration is associated with the uncoiling of an α -helical keratin that unfolds and then incompletely restores with remaining functional defects. These defects are speculated to cause a reduction in water binding capacity causing the clinical symptoms of subsequent roughness and dramatic skin dryness (44). Pretreatment with SLS in the tandem irritation studies allows for more penetration by a second irritant through mechanical skin barrier impairment. However the different responses seen in RA/SLS TEWL compared to SLS/RA tandem experiments is an interesting finding that suggests different molecular mechanism of the 2 irritants. RA appears to decrease skin's susceptibility to SLS effects perhaps by obstructing the percutaneous permeation of SLS (1).

Tandem irritants and percutaneous penetration

Effendy et al. evaluated the permeation of SLS through human skin after pretreatment with RA, and vice versa, by *in vitro* methods (46). Cadaveric skin was prepared for measuring the percutaneous permeation of ^{35}S -labelled SLS and ^3H -labelled RA. It was found that an increase in RA penetration was induced by SLS pretreatment; however, pretreatment with RA did not inhibit the percutaneous permeation of SLS significantly compared to untreated skin ($P > 0.05$). This is in contrast to the previous *in vivo* findings of sequential SLS/RA exposure. Other interactions between the irritants and the skin at molecular or cellular levels may play a role in the different skin responses. This study is a start; much remains to be done.

pH regulation on SC

Exposure of SC to neutralizing buffers and bases has been reported to disturb cutaneous

permeability homeostasis (47). The human skin has long been known to display a protective acidic surface ('acid mantle'), originating from mainly exogenous sources of microbial, sebaceous and eccrine gland origin. Additional endogenous sources that contribute to acidity include the generation of urocanic acid, the secretory phospholipases from free fatty acids from phospholipids, and the activity of a sodium-proton exchanger (48).

Upon analysing the tandem irritation data, we arbitrarily determined our definition of quenching as a tandem TEWL value that is decreased by a factor ≤ 0.5 compared to the expected sum of TEWL of the individual irritants: quenching was observed with lactic acid and acetic acid at pH 2 in tandem with SLS. A proposed explanation could be that the acidic pH allows free fatty acids to assemble more efficiently into ceramides and cholesterol-containing membrane bilayers as proposed by Bouwstra et al. (48) Two enzymes are responsible for this lipid processing, one which hydrolyses glucosylceramides, and another which hydrolyzes sphingomyelin to ceramides; both are most active at an acidic pH (48). These mechanisms may contribute to bilayer lipid membrane stability in the tandem studies that utilized SLS/fruit acids at pH 2, as the acidic pH normalizes SC integrity/cohesion, hence the tandem quenching effect.

Future studies

The result observed in the tandem irritation studies may shed light into the mechanism of action of the various chemicals as they penetrate the SC. Hypotheses on solute permeation and skin barrier impairment are still investigated from different aspects. The various synergistic, additive, and quenching tandem results may be explained by theories of penetration mechanism and compartment interaction. Penetration is governed mainly by molecular size, polarity, melting point, and H-bond capacity of the permeant; all which are contributors to the maximum flux of a solute. Compartment interaction depends largely on an active interaction between cutaneous organelles and the permeating solutes. This was observed in the uncoiling of epidermal proteins, alteration in the quality of SC lipids, and activation of SC zymogens as an effect of acidic exposure. More data from tandem irritation experiments will help deduce the pathogenesis of ICD. Measurement with non-invasive bioengineering methods has provided a controlled environment for data collection. Complex interactions between various irritants remain to be solved hence special

attention is necessary at the workplace and relevant measures should be taken to minimize irritant exposure.

Current information suggests that multiple mechanisms may be involved in the clinical response noted with tandem applications. In spite of this complexity and the intriguing possibility with even more complex chemical mixtures (for instance those in cutting oil), model studies as summarized here provide a difficult but important avenue of investigation to minimize irritant dermatitis. Our hypothesized quenching as suggested by Bouwstra et al. (48) also suggests practical future interventions.

References

- Effendy I, Weltfriend S, Patil S, Maibach H I. Differential irritant skin responses to topical retinoic acid and sodium lauryl sulphate: alone and in crossover design. *Br J Dermatol* 1996; 134: 424–430.
- Ale S I, Laugier J K, Maibach H I. Differential irritant skin responses to topical retinoic acid and sodium lauryl sulphate: II. Effect of time between first and second exposure. *Br J Dermatol* 1997; 137: 226–233.
- Wigger-Alberti W, Krebs A, Elsner P. Experimental irritant dermatitis due to cumulative epicutaneous exposure to sodium lauryl sulfate and toluene: single and concurrent application. *Br J Dermatol* 2000; 143: 551–556.
- Kappes U P, Göritz N, Wigger-Alberti W, Heinemann C, Elsner P. Tandem application of sodium lauryl sulfate and n-propanol does not lead to enhancement of cumulative skin irritation. *Acta Derm Venereol* 2001; 81: 403–405.
- Fluhr J W, Bankova L, Funchs S et al. Fruit acids and sodium hydroxide in the food industry and their combined effect with sodium lauryl sulphate: controlled *in vivo* tandem irritation study. *Br J Dermatol* 2004; 151: 1039–1048.
- Schlemman-Willers S, Fuchs S, Kleesz P, Grieshaber R, Elsner P. Fruit acids do not enhance sodium lauryl sulphate-induced cumulative irritant contact dermatitis *in vivo*. *Acta Derm Venereol* 2005; 85: 206–210.
- Rothenborg H W, Menné T, Sjølin K E. Temperature dependent primary irritant dermatitis from lemon perfume. *Contact Dermatitis* 1977; 3: 37–48.
- Ribaud C, Garson J C, Doucet J, Lévêque J L. Organization of stratum corneum lipids in relation to permeability: influence of sodium lauryl sulfate and preheating. *Pharm Res* 1994; 11: 1414–1418.
- Berardesca E, Vignoli G P, Distanti F et al. Effects of water temperature on surfactant-induced skin irritation. *Contact Dermatitis* 1995; 32: 83–87.
- Øhlenslæger J, Friberg J, Ramsing D, Agner T. Temperature dependency of skin susceptibility to water and detergents. *Acta Derm Venereol* 1996; 76: 274–276.
- Clarys P, Manou I, Barel A O. Influence of temperature on irritation in the hand/forearm immersion test. *Contact Dermatitis* 1997; 36: 240–243.
- Fluhr J W, Bornkessel A, Akengin A et al. Sequential application of cold and sodium lauryl sulphate decreases irritation and barrier disruption *in vivo* in humans. *Br J Dermatol* 2005; 152: 702–708.
- Fluhr J W, Praessler J, Akengin A et al. Air flow at different temperatures increases sodium lauryl sulphate-induced barrier disruption and irritation *in vivo*. *Br J Dermatol* 2005; 152: 1228–1234.
- Fluhr J W, Akengin A, Bornkessel A et al. Additive impairment of the barrier function by mechanical irritation, occlusion and sodium lauryl sulphate *in vivo*. *Br J Dermatol* 2005; 153: 125–131.
- Wigger-Alberti W, Spoo J, Schliemann-Willers S, Klotz A, Wlsner P. The tandem repeated irritation test: a new method to assess prevention of irritant combination damage to the skin. *Acta Derm Venereol* 2002; 82: 94–97.
- Agner T, Serup J. Skin reactions to irritants assessed by non-invasive bioengineering method. *Contact Dermatitis* 1989; 20: 352–359.
- Hutchings C V, Shum K W, Gawkrödger D J. Occupational contact dermatitis has an appreciable impact on quality of life. *Contact Dermatitis* 2001; 45: 17–20.
- Bauer A, Kelterer D, Stadler M et al. The prevention of occupational dermatitis in bakers, confectioners, and employees in the catering trades. Preliminary results of a skin prevention program. *Contact Dermatitis* 2001; 44: 85–88.
- Mathias C G, Maibach H I. Dermatotoxicology monographs I. Cutaneous irritation: factors influencing the response to irritants. *Clin Toxicol* 1978; 13: 333–346.
- Frosch P J, Kligman A M. The soap chamber test. A new method for assessing the irritancy of soaps. *J Am Acad Dermatol* 1979; 1: 35–41.
- Willis C M, Stephens C J, Wilkinson J D. Assessment of erythema irritant contact dermatitis. Comparison between visual scoring and laser Doppler flowmetry. *Contact Dermatitis* 1988; 18: 138–142.
- Pinnagoda J, Tupker R A, Agner T, Serup J. Guidelines for transepidermal water loww (TEWL) measurement. A report from the standardization group of the European society of contact dermatitis. *Contact Dermatitis* 1990; 22: 164–178.
- Nilsson G E, Otto U, Wahlberg J E. Assessment of skin irritancy in man by laser Doppler flowmetry. *Contact Dermatitis* 1982; 8: 401–406.
- Courage W. Hardware and measuring principle: corneometer. In: *Bioengineering of the Skin, Water and the Stratum Corneum*, Elsner P, Berardesca E, Maibach H I (eds): Boca Raton, CRC Press, 1994: 171–175.
- Elsner P. Chromametry. Hardware, measuring principles and standardization of measurements. In: *Bioengineering of the Skin: Cutaneous Blood Flow and Erythema*, Berardesca E, Elsner P, Maibach H I (eds): Boca Raton, CRC, 1994: 247–252.
- Pierard G E. EEMCO guidelines for the assessment of skin colour. *J Eur Acad Dermatol Venereol* 1998; 10: 1–11.
- Fullerton A, Fischer T, Lahti A, Wilhelm K P, Takiwaki H, Serup J. Guidelines for the measurement of skin colour and erythema. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1996; 35: 1–10.
- Rycroft R J. Occupational dermatoses from warm dry air. *Br J Dermatol* 1981; 105 (Suppl. 21): 29–34.
- Veien N K, Hattel T, Laurberg G. Low-humidity dermatosis from car heaters. *Contact Dermatitis* 1997; 37: 138.
- Bauer A, Kelterer D, Stadler M et al. The prevention of occupational contact dermatitis in bakers, confectioners, and employees in the catering trades. Preliminary results of a skin prevention program. *Contact Dermatitis* 2001; 44: 85–88.
- Tacke J, Schmidt A, Fartasch M, Diepgen T L. Occupational contact dermatitis in bakers, confectioners, and cooks. A population-based study. *Contact Dermatitis* 1995; 33: 112–117.
- Halkier-Sørensen L, Thestrup-Pedersen K. The relevance of low skin temperature inhibiting histamine-induced itch to the location of contact urticarial symptoms in the fish processing industry. *Contact Dermatitis* 1989; 21: 179–183.
- Halkier-Sørensen L, Menon G K, Elias P M et al. Cutaneous barrier function after cold exposure in hairless mice: a model to demonstrate how cold interferes with

- barrier homeostasis among workers in the fish-processing industry. *Br J Dermatol* 1995; 132: 391–401.
34. Haftek M, Teillon M H, Schmitt D. Stratum corneum, corneodesmosomes and ex vivo percutaneous penetration. *Microsc Res Tech* 1998; 43: 242–249.
35. Froebe C L, Simion F A, Rhein L D, Cagan R H, Kligman A. Stratum corneum removal by surfactants: relation to in vivo irritation. *Dermatologica* 1990; 181: 277–283.
36. Faucher J A, Goddard E D. Interaction of keratinous substrates with sodium lauryl sulfate. *J Soc Cosmet Chem* 1970; 29: 323–337.
37. Leveque J L, deRigal J, Saint-Leger D, Billy D. How does sodium lauryl sulfate alter the barrier function in man? A multiparametric approach. *Skin Pharmacol* 1993; 6: 111–115.
38. Pirot F, Kalia Y N, Stinchcomb A L, Keating G, Bunge A, Guy R H. Characterization of the permeability barrier of human skin in vivo. *Proc Natl Acad Sci USA* 1997; 94: 1562–1567 31.
39. Elias P M. Epidermal lipids, barrier function, and desquamation. *J Invest Dermatol* 1983; 80 (Suppl.): 44s–49s.
40. Tanojo H, Maibach H I. Percutaneous absorption: 2000. *Curr Probl Dermatol* 2001; 137–140.
41. Potts R O, Guy R H. A predictive algorithm for skin permeability: the effects of molecular size and hydrogen bond activity. *Pharm Res* 1995; 12: 1628–1633.
42. Magnusson B M, Anissimov Y G, Cross S E, Roberts M S. Molecular size as the main determinant of solute maximum flux across skin. *J Invest Dermatol* 2004; 122: 993–999.
43. Fulmer A W, Kramer G J. Stratum corneum lipid abnormalities in surfactant-induced dry scaly skin. *J Invest Dermatol* 1986; 86: 598–602.
44. Wilhelm K P, Cua A B, Wolff H H, Maibach H I. Surfactant-induced stratum corneum hydration in vivo: prediction of the irritation potential of anionic surfactants. *J Invest Dermatol* 1993; 101: 310–315.
45. Goffin V, Pierard-Franchimont C, Pierard G E. Passive sustainable hydration of the stratum corneum following surfactant challenge. *Clin Exp Dermatol* 1999; 24: 308–311.
46. Effendy I, Weltfriend S, Kwangsukstith C, Singh P, Maibach H I. Effects of *all-trans* retinoic acid and sodium lauryl sulphate on the permeability of human skin *in vitro*. *Br J Dermatol* 1996; 135: 428–432.
47. Hachem J, Crumrine D, Fluhr J, Brown B E, Feingold K R, Elias P M. PH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol* 2003; 121: 345–353.
48. Bouwstra J A, Gooris G S, Dubbelaar F E, Ponc M. Cholesterol sulfate and calcium affect stratum corneum lipid organization over a wide temperature range. *J Lipid Res* 1999; 40: 2303–2312.
49. Fluhr J W, Kao J, Jain M et al. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *J Invest Dermatol* 2001; 117: 44–51.

Address:

Francisca Kartono
Department of Dermatology
UCSF
90 Medical Center Way
Suite 110
San Francisco, CA 94143
USA