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B Scanning Evaluation of Irritant Reactions with Binary Transformation and Image analysis

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In order to characterize and quantify irritant skin reactions, 12 women aged 18 to 45 underwent 5 patch tests with sodium lauryl sulfate at the following concentrations: 0.5, 1, 2, 3 and 5%. The tests, applied on volar forearm skin, were removed after a 24-h application. Evaporimetry and B scanning were carried out at the beginning and at 24, 48 and 72 h after patch test application. Echographic recordings were performed by Dermascan C (Cortex Technology, Hadsund, Denmark). After the acquisition, the echographic images were processed by a dedicated software (Dermavision 2D, Cortex Technology, Hadsund, Denmark) enabling the selection of amplitudes of interest and the transformation into a binary colour system. By attributing one colour to a selected amplitude band, part of an image can be highlighted and assessed by a value corresponding to the number of pixels (picture elements). For the evaluation of the images, 2 bands were chosen. The first, ranging from 201 to 255 is able to highlight hyperreflecting parts of the pictures (epidermis, lower part of the dermis); the second, ranging from 0 to 30, marks the hypo-echogenic part of the tissue, which is the site of inflammation.

The evaluation by means of the 201–255 amplitude band showed a marked decrease of the echogenicity of the epidermis which was more pronounced at 24-h determinations and for higher concentrations of sodium lauryl sulphate (SLS), whereas the increase in pixel numbers, shown by the 0–30 band, was slight and apparent only for high SLS concentrations. Echographic data and transepidermal water loss (TEWL) values showed a good correlation. Thus, we can conclude that this evaluation method proved useful in assessing superficial skin damage induced by irritants. *Key words: SLS patch test; Echographic evaluation; Image analysis; Evaporimetry.*

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Irritant contact dermatitis and subclinical skin irritation are common but complex clinical problems. Unlike allergic contact dermatitis, which is always accompanied by subjective and objective signs, skin damage induced by an irritant substance can exist in the absence of clear clinical alterations thus making the skin more vulnerable to further irritation (1). For this reason, instrumental assessment of subclinical irritation is particularly important.

A better understanding of the pathophysiological mechanisms underlying irritant skin damage has been achieved by experimental irritant patch testing. Functional investigation of skin blood flow, skin colour and skin thickness changes, as measurements of the inflammatory process, and of trans-epidermal water loss, reflecting alterations of the barrier function of the stratum corneum, have been performed by many researchers (2–9).

B scanning assessment of allergic patch test reactions has proved a reliable method for their quantification, and is well correlated to clinical scoring (10). We have described an evaluation method employing a new software package to be used on images recorded by a 20-MHz B scanner, enabling the determination of the surface of image areas reflecting within homogeneous echo-amplitudes (11). Using this method, we demonstrated that the number of pixels mirrored within a pre-selected range shows an increase that is proportional to the intensity of a positive allergic reaction.

Our study aims at evaluating irritant reactions using this new method, comparing the results of the echographic assessment with clinical scoring and with values for transepidermal water loss (TEWL), which is considered a reliable method for the evaluation of subclinical water barrier function damage.

MATERIAL AND METHODS

Patients and patch tests

Twelve women, aged 18 to 45 years, underwent patch tests with 0.5, 1, 2, 3 and 5% sodium lauryl sulphate (SLS) (SIGMA Chemical Co., purity according to monograph 95%) with a 24-h application time. A filter paper disk was put into a large Finn Chamber (Epitest, Ltd., Finland) and wet with 30 µl of each solution. After basal recordings, patch tests were applied on the flexural aspect of the left forearm, fixed to the skin by Scanpor tape (Norgesplaster, Finland). Two patch tests were placed, in pairs, 4 cm below the antecubital fossa, two more patches 4 cm lower, the fifth 4 cm below these, at mid distance between the pairs. The positioning of the chambers was systematically varied, but blinded to the examiner. On the distal part of the forearm, a patch test with sterile water and an empty chamber without the paper disk were applied. After patch test removal and between assessments, patch test sites were covered by a light gauze dressing.

Clinical evaluation

Clinical scoring was carried out, before instrumental evaluations, by using a five-point intensity scale, as follows: normal skin = 0; scaling or very weak erythema = 1; weak erythema and slight infiltration = 2; marked erythema, infiltration and vesicles = 3; marked erythema, infiltration, vesicles, abrasion and possibly exudation = 4.

Instrumental assessments

Instrumental assessments were performed after a 30-min acclimatization period, at the beginning of the experiment, after 24 h, 30 min after patch test removal, and after 48 and 72 h. All measurements were carried out on relaxed reclining subjects in a climatic chamber with temperature set at 21–22°C and humidity at 45–50%.

Evaporimetry

TEWL was determined by an evaporimeter (EP1, Servomed, Stockholm, Sweden). This instrument uses the method of water pressure gradient calculation described in detail by Nilsson (12). During all measurements, the probe was hand held using an insulating glove, thus avoiding heating of the probe. After stabilisation of the TEWL value, 30–45 s after application of the probe to the skin, damping filter buttons were pressed in order to smooth fluctuations in TEWL. TEWL value displayed during the next 30-s period was considered the

Table I. *Skin thickness values in mm (± SD) at SLS patch test sites*

Concentration	Baseline	24 h	48 h	72 h
0.5%	1.02 (0.07)	1.12 (0.10)	1.12 (0.11)	1.1 (0.11)
1%	1.01 (0.08)	1.1 (0.10)	1.13 (0.07)	1.19 (0.36)
2%	1.07 (0.09)	1.24 (0.22)	1.29 (0.20)	1.32 (0.42)
3%	1.09 (0.10)	1.22 (0.19)	1.27 (0.17)	1.32 (0.39)
5%	1.1 (0.08)	1.42 (0.28)	1.41 (0.20)	1.38 (0.49)

measured value (13). TEWL measurements were only performed at SLS treated skin sites.

Echographic evaluations

The echographic recordings were carried out using a 20-MHz B scanner (Dermascan C, Cortex Technology, Hadsund, Denmark). This instrument has already been described in detail elsewhere (10). For all recordings a standard swept gain curve at a constant level of 22 dB was used. The evaluations were performed by employing the zoom function in axial direction at the first magnification (at factor 2) which enables exploring of the tissue up to a depth of 6.71 mm. The automatic calculation of skin thickness was based on velocity of sound in skin of 1580 m/s. Assessment of thickness of epidermis and dermis was performed in B mode, outlining the image of the whole skin block on the screen and determining the extension by the region of interest (ROI) function. From this area a mean thickness, based on the values of 224 A scan lines (obtained by dividing the value of the area by 224) can be calculated (14).

Image analysis

After the acquisition, the echographic images were processed by a dedicated programme (Dermavision 2D, Cortex Technology, Hadsund, Denmark), set up on an IBM compatible system (11). This programme has been described elsewhere (15). For the evaluation of the images two bands were chosen. The first, ranging from 0 to 30, marks hyporeflecting areas corresponding to oedema and inflammatory infiltration; the second, ranging from 201 to 255, is able to highlight hyper-reflecting parts of the picture. For every image, pixel values of areas reflecting in these ranges were determined. The chosen regions of interest were, for the 0–30 band, the entire skin block appearing on the screen; for the 201–255 interval, the hyper-reflecting band, corresponding to the epidermis and, separately, the lower part of the dermis.

Statistics

For each SLS dilution and for control tests, TEWL, thickness and pixel values referring to baseline skin and to 24-, 48- and 72-h assessments were compared using the ANOVA test for repeated values and the Student-Newman-Keuls (SNK) test. The Spearman rank correlation coefficient was calculated to study the relationship between the visual grading system and the echographic evaluations. Regression analysis was used to detect linearity between TEWL data and the number of pixels. Finally, the Student's *t*-test for paired values was used, to compare baseline values of pixels to values obtained from SLS treated skin sites, which had been scored with 0.

RESULTS

Clinical evaluation

Out of 180 evaluations of patch test reactions (12 patients × 5 patch tests × 3 times of assessment), 112 resulted in a positive clinical score. Forty-nine scored 1, 34 scored 2, 17 scored 3 and 12 scored 4. No positive reactions were detected at control test sites. Reactions were at their maximum intensity at 24 h: for each test area, the total score was at its highest at 24-h assessment and decreased progressively at 48 and 72 h. The reaction,

Fig. 1. Images of normal skin of the volar forearm (left upper corner) and of patch test reactions of different degrees of positivity, after selection of the 0–30 amplitude band and binary transformation. The area reflecting in the selected interval appears green in the dermis and increases according to positivity degree.

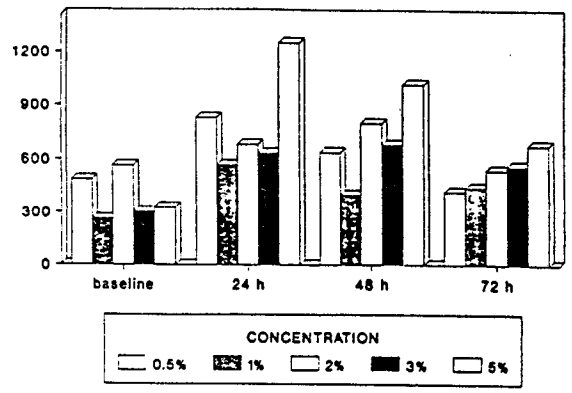

Fig. 2. Number of pixels forming areas reflecting in the 0–30 amplitude interval at SLS patch test sites, performed with 0.5% to 5% SLS in distilled water, assessed at different times. Number of pixels is at its maximum at 24-h determinations.

as assessed clinically, was proportional to the concentration of the irritant solution.

Echographic evaluation

Skin thickness, as assessed in B mode, showed an increase in time which was more evident with high SLS concentrations (Table I). The increase was at its maximum at 24 h for 0.5 and 5% concentrations, and at 72 h for 1, 2 and 3% SLS patch tests. Comparing consecutive times of assessment, differences in skin thickness values were significant only for 0.5 and 5% SLS concentrations. At a level of significance of 0.05 it was possible to distinguish between baseline values and 24-, 48- and 72-h values.

Processing by the 0–30 band showed an increase in the extension of the area reflecting in this range for all dilutions (Fig. 1). The highest increase was observed for the 5% solution. For all concentrations, the increase was more evident at 24-h determinations and values dropped again after that time (Fig. 2). Statistical differences between pixel values, assessed at different times for the same SLS dilution, were observed for 3 and 5% patch tests. SNK evaluation showed significant differences between baseline values and those assessed at 24, 48 and 72 h for 3% patches, and differences between baseline and 24- and 48-h values and between 48- and 72-h ones for 5% patch tests. The correlation coefficient r between number of pixels and visual scoring was 0.432.

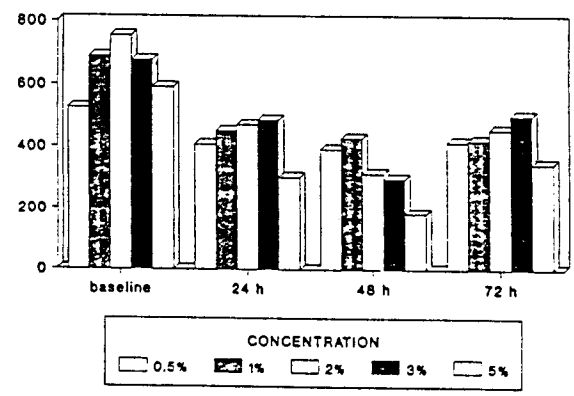

Fig. 3. Number of pixels forming areas reflecting in the lower dermis in the 201–255 amplitude interval, at sites of SLS patch tests, performed with 0.5% to 5% SLS in distilled water, assessed at different times. Pixel values decrease especially at 5% SLS patch tests.

Dermis evaluation by the 201–255 band showed a slight decrease at all SLS treated skin sites and at all times, which was more evident at 48-h assessment (Fig. 3). This decrease reached statistical significance for 2 to 5% patch tests. For 5% patch tests, at a level of significance of 0.05, it was possible to distinguish between baseline values and 24-, 48- and 72-h values.

Considerable information could be obtained by processing with the 201–255 band to highlight areas reflecting in the upper part of the skin (Fig. 4). Pixel values showed a marked decrease for all concentrations especially at 24-h determinations. This decrease was more pronounced for patches performed with 3 and 5% solutions (Fig. 5). Variations in pixel numbers were statistically significant for 1 to 5% patches. Differences between groups were detectable for 1 to 5% solutions between baseline values and 24- and 48-h values and between 24- and 72-h values; moreover, for the 2% concentration, differences between baseline and 72-h and between 48- and 72-h values were evident. Finally, 3% patch test determinations differed between 48-h and 24- and 72-h measurements.

No significant variations were observed for values of echographic measurements at control test sites.

The correlation coefficient r between the echographic evaluation by the 201–255 band and the visual grading system was −0.277. In view of this poor correlation, values of pixels reflecting in the 201–255 range of SLS treated skin sites scoring 0 were compared to baseline values for the same skin site by the Student's t-test for paired values. This test showed a highly significant difference between the 2 groups ($p < 0.001$).

Evaporimetry

TEWL at SLS treated sites had significantly increased at 24 h compared to basal skin. Readings fell gradually, but the increases were still significant at 48 and 72 h. Increases were more evident for the highest SLS concentrations (Fig. 6).

A significant linear correlation was demonstrated between TEWL values and number of hyper-reflecting pixels (201–255) in the epidermis. The linear regressions were as follows: $y = -0.06 \times +29.19$ ($r = -0.832$).

DISCUSSION

It is well known that surfactant induced changes are present after applications of the irritant even though the skin appears normal from a clinical and a subjective view point. Moreover, it has been demonstrated that the inflammatory response associated with skin barrier function damage induced by SLS is correlated with the concentration of SLS (8). In fact, even in the absence of clinical signs of skin irritation, increases in TEWL were detected before increases in skin blood flow, sustaining the view that barrier function damage precedes the inflammatory process (7, 16). Thus, TEWL and other correlated dynamic parameters, such as skin surface water loss, have proved to be reliable methods for the assessment of skin irritation.

For this reason, we chose the TEWL measurement as a

Fig. 4. Images of normal skin of the volar forearm (left upper corner) and of patch test reactions of different degrees of positivity, after selection of the 201–255 band and binary transformation. Area reflecting in the selected interval appears green. Epidermis echo and echogenicity of the lower dermis are clearly decreased at positive patch tist sites compared to normal skin.

method which is both comparable to clinical scoring and to echographic data referring to subclinical irritation.

Our results confirm a positive dose response correlation between irritant doses of SLS and skin damage as assessed clinically. TEWL results are in accordance with previous data referring to irritant patch tests performed with a 24-h application time (7, 16, 18).

The increase in skin thickness, already evidenced by Serup and co-workers using a 15 MHz A scan equipment (19), was confirmed by our B scan assessments which showed that this rise, which is tied to the inflammatory process, reaches its highest level later than TEWL value evaluating skin barrier damage.

Our image analysis procedure, based on the selection of amplitude intervals of interest and on the determination of the values of the pixels forming areas reflecting within the chosen amplitude bands, enables a numerical description of physiological and pathological conditions of the skin. The choice of the bands for evaluating irritant skin reactions was based on a previous experience concerning assessment of allergic patch test responses (11).

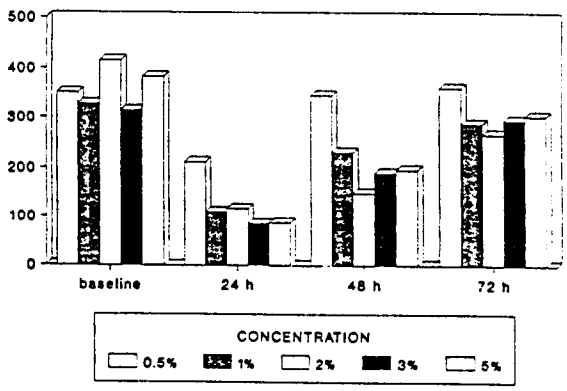

Fig. 5. Number of pixel forming areas reflecting in the upper part of the skin in the 201–255 interval at sites of 0.5% to 5% SLS patch tests, assessed at different times. Pixel values decrease at 24-h determinations.

The echographic aspect of an irritant reaction is characterized by the presence of a lower density and a greater homogeneity of the dermis corresponding to a decrease in reflectivity as in allergic reactions. Unlike the latter, irritant reactions show a clear decrease in the epidermis echo which is hyporeflecting and interrupted or has totally disappeared.

Thus, an evaluation interval covering the lowest amplitudes can quantify the appearance of dermal hyporeflectivity, whereas a band comprising the highest amplitudes is helpful in determining the damage of the entrace echo. Data calculated by the 0–30 band evaluation show a fair correlation to clinical scoring, whereas reflectivity of the epidermis, as assessed by the 201–255 band, is inversely proportional to TEWL values. The significance of the superficial hypo-echogenicity is difficult to explain. It is interesting to observe that it is proportional to functional damage, as demonstrated by TEWL, and could be considered as a visual equivalent of impaired water barrier function of the skin.

Moreover, this method seems very sensitive and is therefore useful in evaluating subclinical irritation. In fact, SLS treated skin areas which had been clinically evaluated as negative test showed echographic modifications which could be quantified by image analysis and were significant in comparison to normal skin values.

Compared to other non invasive techniques in assessing irritation, the echographic evaluation has the advantage of a quick execution. In fact, no pre-conditioning of the subjects is necessary, whereas subjects have to rest for at least 15 min

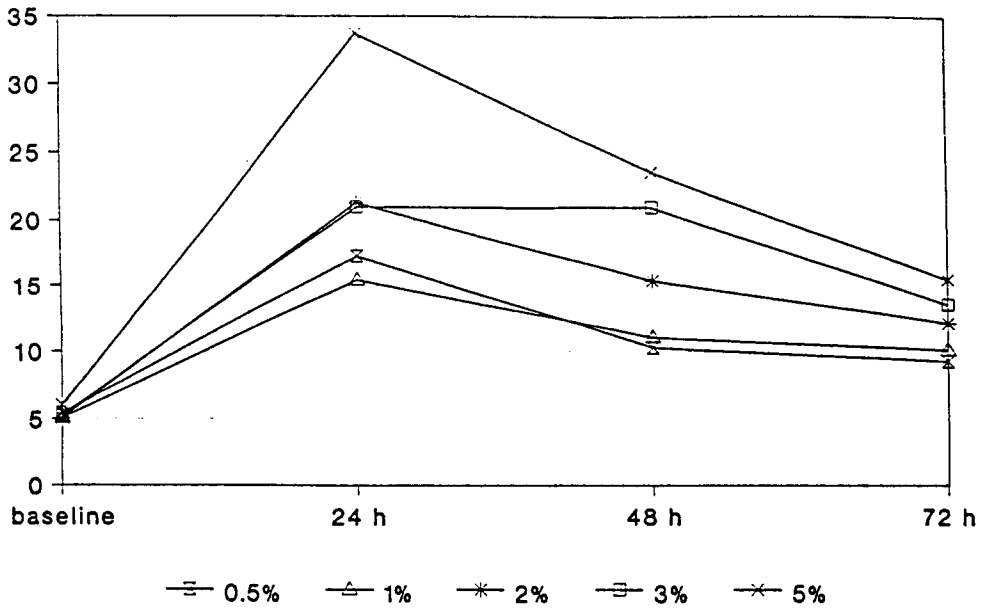

Fig. 6. TEWL average values assessed at different times at 0.5% to 5% SLS patch test sites. The increase in TEWL reaches its highest level at 24-h assessment.

before measurements of TEWL, skin colour and skin blood flow.

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