Prediction of Skin Irritation by Noninvasive Bioengineering Methods

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Keywords

Atopic dermatitis · Corneometer · Laser Doppler flowmetry · Natural moisturizing factor (NMF) · Profilaggrin · Soap effect · Spectrophotometry · T-helper-2 polarization · Visual scoring

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1 Core Messages

- The ultimate goal of skin irritancy testing is to understand and predict chronic irritant contact dermatitis. Visual scoring constitutes the basis of evaluating skin irritancy testing.
- By using noninvasive bioengineering methods, various aspects of skin irritation and inflammation can be quantified, both in subclinically and clinically involved skin.
- The result of irritancy testing may depend upon the type of testing model in which compounds are brought into contact with the skin (onetime occlusive patch test, repeated occlusive test, or repeated open tests). The model of choice depends on the presumed circumstances under which these compounds are met with in daily life.
- Great interindividual variations in susceptibility to irritants are recognized among healthy nonatopic subjects. The role of barrier function, adaptation capacity, and other factors that may determine susceptibility is discussed.
- Atopic dermatitis is unique in its high reactivity. New findings point toward the role of filaggrins in lowering the barrier function in a subgroup of atopic dermatitis patients. Furthermore, characteristic stratum corneum lipid patterns have been identified.
- Prospective studies have identified history of hand dermatitis and atopic dermatitis as important personal risk factors for the development of hand dermatitis in high-risk occupations.

2 Introduction

Chronic irritant contact dermatitis (ICD) is a frequently occurring skin disease in many occupations in which workers are exposed to irritants. High-risk occupations include hairdressing, health care, cleaning, and metal working. Chronic ICD is located mainly on the hands and forearms. The prognosis is poor, and persistent chronic ICD may result in impaired quality of life and loss of work (Meding et al. 2005). It is therefore of great importance to find ways to lower the incidence of chronic ICD. In this process, it is crucial to have insight into the factors that can predict irritancy. Whether or not chronic ICD will develop depends on the balance between the sum of all extrinsic, harmful factors (irritants in the work and home environment, low humidity and high temperature, etc.) inflicted on the skin on the one hand and intrinsic, constitutional factors regulating the reparative capacity of the skin on the other hand (Hagerman 1957). Predictive irritancy testing as a method to understand and predict chronic ICD should be based on the etiological concepts of chronic ICD. Therefore, considering predictive irritancy testing, it is helpful to make the same division into extrinsic and intrinsic factors, yielding two main work categories: (1) predictive irritancy testing of various substances aimed to select the least irritant substance and (2) predictive irritancy testing with one or more standard irritant(s) aimed to select a population that is at risk of developing chronic ICD. Prediction of risk of chronic ICD is done using human skin irritancy tests in the laboratory and in the occupational setting. Both strategies will be covered in this chapter.

In finding new chemicals and finished products with low irritant potential, the industry making such products has increasingly to rely on human skin models, as animal models are nowadays abjured on ethical grounds and because these methods had been proved to be poorly predictive in man (Phillips et al. 1972). In order to avoid testing on humans as much as possible, in vitro tests have been developed. It can be expected that in vitro testing will be used more extensively in future for screening chemicals with low irritancy. This tendency toward in vitro testing will not abrogate the need for in vivo tests on humans, which will remain the "gold standard" of predictive irritancy testing, at least for the near future.

Evaluation of the responses is an important feature of skin irritancy testing. Besides visual scoring, there are several noninvasive bioengineering methods, each describing a particular aspect of skin irritancy. In this chapter, the most commonly used techniques are described: transepidermal water loss (TEWL), laser Doppler flowmetry (LDF), skin color/erythema, and skin hydration. The chapter starts with a description of the characteristics of each of these evaluation methods,

followed by a discussion on studies aimed at investigating their power to evaluate irritant responses by various types of irritants. In the next paragraphs, several factors are discussed which can influence the outcome of in vivo irritancy testing in humans, classified as extrinsic and intrinsic factors. The subject is not treated exhaustively; for a more complete survey on factors influencing irritant reactions, the reader is referred to the guidelines on sodium lauryl sulfate (SLS) exposure tests, a report from the Standardization Group of the European Society of Contact Dermatitis (ESCD) (Tupker et al. 1997a). These parts are followed by a paragraph on predictive testing in the occupational setting, aimed at identifying subjects who are at risk of developing chronic ICD when starting their career in a high-risk occupation. In the final paragraph, general concluding comments are given on the subject of predictive testing.

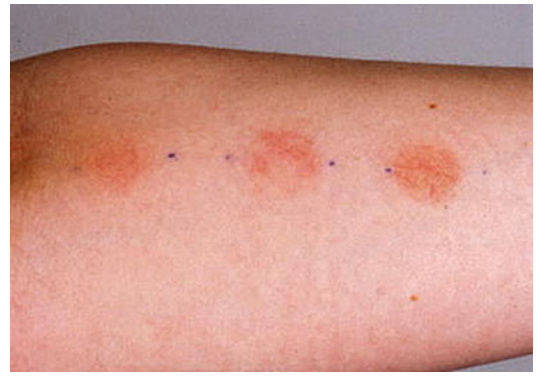

Fig. 1 Forearm of a volunteer on day 19 after repeated occlusive exposures to sodium secondary dodecan sulfonate (SDS) (most *left*), sodium dodecylbenzenesulfonate (DBS) and SLS (most *right*). Visual scores for SDS: erythema, moderate (2); roughness, slight (1); scaling, minute flakes (1); edema, none (0); fissures, none (0). Visual scores for DBS: erythema, 2; roughness, 1; scaling, 1; edema, 0; fissures, 1. Visual scores for SLS: erythema, 1; roughness, 2; scaling, 3; edema, 1; fissures, 2

3 Noninvasive Evaluation Methods

3.1 Visual Scoring

The ultimate goal of skin irritancy testing is to understand and predict chronic ICD, which is of course a clinical condition. Therefore, visual scoring constitutes the basis of evaluating skin irritancy testing. The significance of visual scoring is best exemplified by the description of the morphology of SLS reactions by Björnberg (1968). Erythema, sometimes associated with infiltration and sometimes with superficial erosion of the epithelium, is the main feature of acute reactions. With higher concentrations, vesicular and even pustular reactions may be seen. During healing of acute reactions, scaling and fissuring will take over. The same appearance with erythema, scaling, and fissuring is seen during repeated application of SLS (Tupker et al. 1989a) (see Fig. 1). The so-called soap effect or *effet de savon* consists of a fine wrinkled surface contour associated or followed by chapping, the latter representing a characteristic roughening of the skin. This characteristic soap effect may be observed when SLS at low concentration is used.

The clinical appearance of acute and cumulative reactions differs. The ESCD has therefore developed scoring systems to grade acute and subacute/cumulative SLS irritant reactions (Tupker et al. 1997a). In both cases, erythema, roughness/contour, scaling, edema, and fissures are graded into scores from 0 to 3. From these separate scorings, an estimate of the overall intensity of the reaction can be drawn. When this subdivision into the a qualities of irritation is not necessary, a simple scoring system is available. These visual scoring systems, which are designed for SLS, can also be used for other types of irritants.

3.2 Transepidermal Water Loss (TEWL)

Measurement of TEWL is used in many research centers for studying the water barrier function of the skin. In this chapter, attention is focused on the open chamber, gradient estimation method of the commercially available Evaporimeter EP1/EP2 (ServoMed, Stockholm, Sweden), Tewameter (Courage and Khazaka, Cologne, Germany), and DermaLab (Cortex Technology, Hadsund, Denmark).

The total amount of water vapor passing the skin can be divided into water vapor passing the stratum corneum by passive diffusion and water vapor loss as a result of sweating (Rothman 1954). Originally, the term "transepidermal water loss" was applied to indicate the amount of water vapor passing the stratum corneum by passive diffusion (Rothman 1954). However, nowadays "TEWL" refers to the total amount of water loss through the skin. Therefore, it must be kept in mind that TEWL is a reflection of stratum corneum barrier function for water only when there is no sweat gland activity.

In the probe head, two sensors are located at different distances to the skin surface. Each sensor measures the water vapor pressure. The calculated difference in the vapor pressure at the two fixed heights of measurement is the estimated vapor pressure gradient. From this gradient, the evaporative TEWL value, in g/m^2h, is calculated by the signal processing units in the probe handle and/or main unit and digitally displayed. This estimation is valid only within this boundary layer its depth depending on the site, air speed, and convections, forced and/or free (Nilsson 1977). In the absence of convection currents or drafts, a mean depth of about 10 mm may be assumed for this boundary layer.

TEWL measurement is a very delicate technique for which it is of utmost importance to be aware of the many pitfalls in the methodology of measurement and interpretation of the results, in order to achieve reliable outcomes. There are many variables that can influence measurement outcomes, i.e., instrument-related, environment-related, and individual-related variables. Therefore, researchers should adhere to the guidelines published on this subject (Pinnagoda et al. 1990).

3.3 Laser Doppler Flowmetry (LDF)

LDF is an optical technique for estimation of microcirculation based on the Doppler principle. It is suited to determine the degree of superficial blood circulation in irritant reactions characterized by redness. There are several types of LDF instruments using different laser wavelengths and varying measuring areas (Bircher et al. 1994). In experimental dermatology, the most known instruments are the PeriFlux System 5000 and older versions (Perimed, Jarfalla, Sweden).

With LDF, the amount of moving particles (erythrocytes) in the subepidermal plexus can be determined. Monochromatic light is guided by an optical fiber to the skin surface, where reflection, transmission, absorption, and scattering occur. It permeates to a depth of 0.5–1.0 mm. A portion of the backscattered light is picked up by a pair of fibers guiding the light to photodetectors. By signal processing, an output signal that is linearly related to flow, for low and moderate flow rates, is obtained. Since the cutaneous microcirculation is a dynamic system with many functions, e.g., thermoregulation and metabolism, many environmental and individual factors influence the cutaneous blood flow. LDF measurement has a rather high intraindividual coefficient of variation and an even higher interindividual coefficient of variation (reviewed in Bircher et al. 1994). Due to the presence of arteries, arterioles, capillaries, venules, and veins, regional variations are wide. The same holds true for temporal variations, since changes during the cardiac cycle can be visualized, but also slower rhythms (5–10 per min) and circadian rhythms (Yosipovitch et al. 2004). For more details about variables that are individual-, environment-, and technique-related, the reader is referred to the guidelines on this topic (Bircher et al. 1994). Because of the large variations depending on site, a new method has been developed, laser Doppler perfusion imaging (Fullerton et al. 2002). In this technique, the laser beam position is controlled in the X and Y directions, from which a digital image is composed of numerous single-point recordings forming a two-dimensional flow map over an extended skin surface.

3.4 Skin Color/Erythema

Erythema as a result of inflammation can also be evaluated by skin color measurement (colorimetry). Two types of measuring principles are available for this purpose, each of them translating the subjective impression of color in a different way

(Fullerton et al. 1996). A so-called tristimulus colorimeter analyzes the amount of blue, red, and green lights reflected from the skin. A widely accepted system to evaluate color in place of human perception has been developed by the Commission Internationale de l'Eclairage (CIE) (reviewed in Fullerton et al. 1996). In this so-called CIE L *a *b * color space, "L *" describes brightness, "a *" is the red-green chromaticity coordinate, and "b *" is the blue-yellow chromaticity coordinate. The Minolta Chroma Meters (Osaka, Japan) of the 200 and 300 series are instruments based on this tristimulus measuring principle. They consist of a measuring head that emits white light by means of a xenon arc lamp. The color of the reflected light is analyzed by three photocells that measure the amount of blue, red, and green lights. The signals are transmitted to a control unit for processing.

The other measurement principle is by spectrophotometry. Hemoglobin and melanin are the two dominant chromophores of the skin. Hemoglobin shows specific absorption of light in the green range. With increase in erythema, a greater amount of green light is absorbed and less is reflected. The instrument, the DermaSpectrometer (Cortex, Technology, Hadsund, Denmark), is a handheld device that functions as a reflectance photometer using green and red light-emitting diodes as light sources. A silicon photodiode detects the light remitted from the skin. From the intensities of the reflected red and green lights, the "erythema index" is calculated.

The coefficients of variation for a * values were low (Fullerton et al. 1996). Variations may be caused by diurnal fluctuations and by body site. For details concerning sources of variations, further reading of the guidelines on this subject is advised (Fullerton et al. 1996).

3.5 Skin Hydration

The final noninvasive method used for quantitative evaluation of skin function is measurement of skin hydration. This method has become popular due to its low cost and ease of use. It is used to assess the degree of skin dryness in both normal and diseased skin and to monitor the influence of (cosmetic) products on skin dryness. There are different types of instruments estimating skin hydration (Berardesca 1997). They differ with respect to frequency and measuring depth. The Corneometer 820–825 (Courage and Khazaka, Cologne, Germany) operates at low frequency (40–75 Hz). It estimates the water content to a depth ranging between 60 and 100 μm. The Skicon-200EX (I.B.S. Co, Hamamatsu, Japan) measures at high frequency (3.5 Mhz) and more superficially than the Corneometer. Both techniques give only relative indications of water content. In general, the Corneometer is more sensitive when assessing relatively dry skin, whereas the Skicon is more useful in higher water content levels. For reliable results, it is important to ensure an effective standardization of many variables, such as skin temperature, sweat gland activity, anatomical site, etc. Further reading on these influencing factors is necessary before using this method in practice (Berardesca 1997).

3.6 Use of Noninvasive Evaluation Methods in Human Skin Irritancy Models

Detergents and solvents exert a damaging influence on the stratum corneum, and therefore TEWL measurement is an appropriate noninvasive method to evaluate their effects. Using TEWL measurement, differences between detergents with respect to their irritant potency were demonstrated (Tupker et al. 1989a) (see Fig. 2).

A significant linear relationship between dose of SLS and skin response was found for patch tests evaluated by TEWL, LDF, and skin color (Agner and Serup 1990). However, the sensitivity was highest for TEWL, followed by LDF, in its turn followed by skin color measurement.

Similar observations were made in another investigation comparing the use of TEWL and LDF in the evaluation of subclinical and pronounced reactions by SLS (Aramaki et al. 2001a). TEWL appeared the most appropriate method in the lower range of the dose and

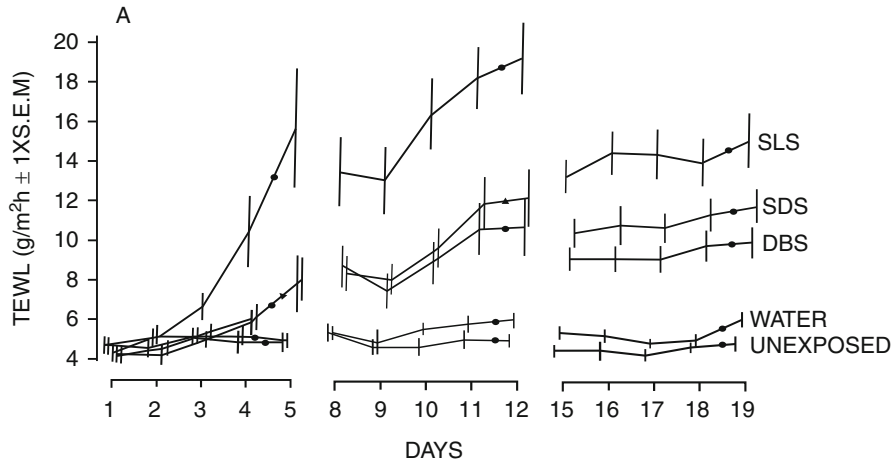

Fig. 2 Mean ± standard error of the mean for TEWL measurement at different sites on the forearms during 3 weeks of exposure to SLS, sodium secondary dodecan sulfonate (SDS), sodium dodecylbenzenesulfonate (DBS), and water

duration, whereas LDF shows clear linear responses in the higher range.

The time course after irritation by dithranol, tretinoin, SLS, tape stripping, and ultraviolet light has been investigated with the aim to study the discriminating power of visual scoring, TEWL, skin color measurement, LDF, and skin hydration (Fluhr et al. 2001). LDF and skin color measurements were the methods of choice when the irritation is determined by an inflammatory reaction with elevated dermal perfusion, clinically visible as redness. In contrast, for insults that exert their damaging effect by barrier function impairment, TEWL and skin hydration were the best methods.

Responses after open exposures to six antiseptics have been evaluated by means of visual scoring, TEWL, LDF, and hydration measurement (Tupker et al. 1997b). Sodium hypochlorite turned out to be the most irritative agent, followed by iodine 1% in ethanol 70%, as evaluated with all methods. The other antiseptics did not induce visible skin changes. With LDF, no signals could be detected either, whereas TEWL and hydration measurement had some discriminative potency. The two most irritative agents caused the same ranking order in all methods, which may be explained by the fact that their irritant mechanism includes several aspects of irritation.

Skin hydration measurement was the only method capable of detecting changes after repetitive patch tests to ethanol, 1-propanol, and 2-propanol (Löffler et al. 2007). No significant changes could be observed regarding erythema (Chromameter CR 300) and TEWL measurement. When the alcohols were applied in a so-called tandem test design, together with SLS, TEWL and erythema revealed significant responses, whereas no significant change was found for skin hydration. Repetitive washing the forearms with SLS induced more skin changes in all testing methods than disinfection with ethanol.

4 Extrinsic Factors that Determine the Outcome of Human Skin Irritancy Models

4.1 Duration of Exposure in the Onetime Patch Test

For many years, closed patch testing has been the favored test method, for both practical and traditional reasons. The 24-h patch test is used in most studies, and applied on the forearm, it may be considered as the "gold standard" for onetime exposure tests (Tupker et al. 1997a).

SLS has been applied for 48 h on the back and forearm in order to combine irritancy testing with routine allergic patch testing (Löffler et al. 2001). Visual scores were more pronounced on the

forearm than on the back, but the scores on the back and forearm showed a close correlation. Visual scores on the forearm correlated well with TEWL values on the forearm, whereas visual scores on the back and TEWL values on the forearm had a poor correlation. In another study by the same group, the interrelationship between the concentration of SLS and exposure time was determined (Aramaki et al. 2001b). There was a clear distinction between the concentrations used only at 12-, 24-, and 48-h exposures. The factor of concentration had a greater impact on the outcome of the irritant response than the factor of exposure duration. To achieve the same irritant response as found with a doubled SLS dose, the application time must be tripled.

The biological effect of a substance depends on the amount of molecules (molarity) rather than the total mass of the molecules (concentration) (Mathias 1983). Therefore, when the purpose of predictive testing is comparison of irritant potential between various compounds, these compounds should be tested on the basis of equal molarity; otherwise, a false impression may be obtained regarding differences in irritancy. This theoretical consideration has practical limitations when mixtures of more than one compound are to be tested, such as commercially available products.

A simple 4-h patch test has been designed to examine crude differences in the effects of SLS on the skin, evaluated by a simple visual scoring system (Basketter et al. 1996). When attempting to use this method to detect variation in susceptibility between summer and winter seasons, no significant differences were found, although there was a trend toward a greater susceptibility in winter. These differences were noted only for extremely high SLS concentrations (20% and 100%).

4.2 Repeated Exposure Models

Although the onetime occlusive patch test is a favored test method because of its ease and rapidity to perform, the disadvantage of this technique is the fact that it mimics only an acute irritant reaction. The more common situation in real life is the development of chronic ICD resulting from multiple repeated exposures. In order to address this issue, the following test protocols have been used: repeated occlusive tests, repeated open tests, and wash tests.

4.2.1 Repeated Occlusive Tests

Kligman and Wooding developed a test method in which substances were repeatedly brought into contact with the skin under occlusion 24 h a day for 20 consecutive days, in order to be able to test substances with a very low irritant potential (Kligman and Wooding 1967). Another way to test compounds of expected low irritancy is the *chamber scarification* technique, in which the skin is scratched before closed patch tests are performed, repeatedly daily for 3 days (Andersen 1996). Using this method, in which the stratum corneum was bypassed, interindividual variation has still been found, demonstrating that a subject's susceptibility to irritants is also dependent on targets underneath the stratum corneum. Frosch and Kligman have used an exposure model in which a substance is applied repeatedly during five consecutive days; the first day for 24 h and the following days for 6 h a day (Frosch and Kligman 1979). To reflect better the conditions in daily practice, models have been developed in which detergents were applied in a *multiple repeated short-time occlusive* way, namely, two times daily 45 min for 1 week (Tupker et al. 1989b, 1995) or 3 weeks (Tupker et al. 1989a, 1990a, 1999). Using this test method, it was possible to rank detergents according to their irritant potential, SLS being by far the most irritant detergent (Tupker et al. 1989a, 1999).

4.2.2 Repeated Open Tests

A further step toward a better reflection of daily life conditions may be the *repeated open exposure* technique. In this method, solutions are applied once daily on the forearm skin using a glass or plastic ring during 20 or 45 min for 1 week (Van der Valk and Maibach 1989; Tupker et al. 1997c) or longer time periods (Tupker et al. 1999; Frosch et al. 1993). Clear differences in the irritancy of various detergents have been demonstrated by this method (Tupker et al. 1997c, 1999). By means of a repetitive open irritation test, it was possible to test the efficacy of different barrier creams

(Andersen et al. 2006). Nonanoic acid 20% and SLS 1% were used as irritants (Andersen et al. 2006). It was shown that nonanoic acid was better than SLS in distinguishing between the protective effects of different "anti-irritants."

4.2.3 Wash Tests

Twice daily 30 min *immersions* of various detergents induced different degrees of erythema and scaling, and SLS was again shown to be the most irritant (Smeenk 1969). The *wash test* represents another open test model, in which the act of washing is mimicked in a repetitive way (Löffler et al. 2007; Imokawa and Mishima 1979; Frosch 1982). Wash tests demonstrated that alcohol exposure caused less skin irritation than detergent exposure (Löffler et al. 2007). Repeated patch testing with these agents was less discriminative.

A few studies have compared the patch test with the wash test with respect to the irritancy potential ranking order of a series of detergents (Smeenk 1969; Imokawa and Mishima 1979; Frosch 1982). In one study (Imokawa and Mishima 1979), discrepancies were found in the ranking order obtained by the two techniques, whereas in the other study (Frosch 1982) similar rankings were found. The wash test, however, appeared to have the greater power of discrimination (Frosch 1982). Comparing the onetime patch test with the immersion test, there was a relatively close correlation between these models, with the exception of soap, which was irritant in the patch test but not in the immersion test (Smeenk 1969). The same finding has been observed in a study investigating four different detergents using onetime occlusive, repeated occlusive, and repeated open tests (Tupker et al. 1999). Only in the onetime occlusive test did soap induce more erythema than the other detergents, whereas in the other models, SLS and sodium cocoyl isethionate had higher visual scores than soap. By means of TEWL measurement, however, the mean ranking order of the detergents was similar in all models (Tupker et al. 1999). The fact that soap was the most irritant substance in the onetime patch test could be explained by the earlier observation that the pH of a soap solution decreased after prolonged contact with the skin, as is the case under 24-h occlusive conditions (Blank and Gould 1961). Soap with a low pH (7.5) induced erythema and pruritus, which did not occur at higher pH (9.5) (Blank and Gould 1961). In another study on the effects of occlusion, it was found that postexposure occlusion by a plastic wrap caused more severe irritation compared with unoccluded exposures to SLS (Van der Valk and Maibach 1989). The degree of augmentation of the irritant response by occlusion differs between varying types of substances. The irritant effect of polar compounds is less influenced by occlusion than that of nonpolar compounds (Treffel et al. 1992).

Hence, it appears that the type of exposure may influence the outcome of the ranking order in irritancy testing. The central question is the following: which exposure method offers the best prediction of real-life exposure? Which poses another question: what are the real-life conditions encountered? In most in-use situations, the uncovered skin is exposed to irritants several times daily. In other situations, already exposed skin is covered by "protective" gloves or other impermeable materials. In the first situation mentioned, the repeated open test seems the best way to simulate this. In the second situation, however, the onetime or repeated occlusive test may be preferred.

Which conclusions can be drawn from the literature on prediction of irritancy? The most reliable test methods are still the human models in which compounds are brought into contact with the skin in an occlusive (onetime 24 h or repeated) or open (repeated) way. The model of choice (occlusive vs open) depends on the presumed circumstances under which these compounds are met with in daily life. One way to refine the human test methods is to assess the test responses by means of noninvasive bioengineering methods. By using these methods, different aspects of skin irritation/inflammation can be quantified, both in subclinically and clinically involved skin. When using noninvasive methods, guidelines on this matter should be adhered in order to achieve trustworthy results. When authors decide not to follow a particular aspect of the guidelines, they should make this clear by stating that they have not adhered to the guidelines in this respect and also give the reason for this. Noninvasive methods should never be a goal per se and always be accompanied

by a reliable visual scoring system (Tupker et al. 1997a). On the other hand, visual scoring has its limitations, such as in dark-skinned persons, in whom detection of erythema is more difficult.

5 Intrinsic Factors that Determine the Outcome of Human Skin Irritation Models

5.1 Preexposure Barrier Function

Using TEWL after 4 days of repeated exposure to SLS as a measure of susceptibility, a close correlation with preexposure TEWL has been found ($R = 0.71$) (Tupker et al. 1989b). A similar correlation coefficient has been noted between TEWL values before and after a single 24-h SLS exposure (Pinnagoda et al. 1989). Subsequent studies have confirmed the close correlation between pre- and postexposure TEWL in the onetime SLS exposure model (Agner 1991). In persons with a high preexposure TEWL, the barrier function is apparently impaired from the start. This allows the irritant to penetrate and damage the skin more easily, which renders the skin more susceptible, particularly in the initial phase of skin irritation. In other studies using onetime occlusive tests, these results could not be confirmed; no correlation (Freeman and Maibach 1988) or poor correlation (Berardesca and Maibach 1988) between pre- and postexposure TEWL values has been found. In long-term repeated exposure models also, lesser correlations between pre- and postexposure TEWL have been noted ($R = 0.31$) (Tupker et al. 1990a). After multiple repeated exposures, the skin will adapt to a certain degree (see below). Therefore, skin reactivity after multiple exposures is probably particularly determined by the ability to develop adaptation, and the influence of preexposure barrier function diminishes (Tupker et al. 1990a). It must be stressed that these reported correlation outcomes have been found for SLS only. Extrapolation to other types of irritants may yield only poor correlation values, since those irritants might penetrate and attack the stratum corneum barrier in a different way (Fluhr et al. 2001).

5.2 Concomitant (Atopic) Dermatitis in Another Body Region

It has been proven that existing dermatitis, irrespective of which type, in another location of the body may enhance reactivity to various irritants (Björnberg 1968). The validity of this phenomenon has been confirmed for atopic dermatitis (AD) (Tupker et al. 1995). AD patients with more severe dermatitis exhibited higher levels of reactivity. In another study, only those patients with active hand eczema had an increased susceptibility to SLS on the upper arm, as opposed to chronic and healed eczema patients and normal controls (Agner 1991). The cause of this proneness to hyperreact to chemicals in the presence of dermatitis elsewhere is not yet known. Shahidullah et al. have observed barrier function impairment on the uninvolved skin sites, related to the severity of dermatitis on other body locations (Shahidullah et al. 1969). Furthermore, TEWL of uninvolved sites of the forearm in patients with acute ICD of the hands was higher than that in patients with healed ICD of the hands, who in turn had higher TEWL values than controls (Effendy et al. 1995). Since the type of dermatitis present did not appear to influence the reactivity, the enhanced susceptibility noted was regarded as secondary to dermatitis activity per se (Gloor et al. 1985). However, Van der Valk et al. have found a significantly elevated TEWL before and after SLS exposure only in the subgroup of patients with a manifest AD and not in manifest ICD (Van der Valk et al. 1985). In a study on patients with a history of AD and patients with a history of contact dermatitis, higher preexposure and postexposure TEWL values have been noted only in patients with a history of AD (Tupker et al. 1990a). It was concluded that the lower preexposure barrier function in AD was at least in part responsible for the enhanced irritant susceptibility in AD, besides other constitutional factors, such as elevated reactivity of cellular targets underneath the stratum corneum, and adaptation capacity (Tupker et al. 1990a). In other studies, patients with active and those with inactive AD reacted more strongly to SLS as compared with

nonatopic controls (Tupker et al. 1995; Nassif et al. 1994). Hence, AD may be regarded as unique in its high skin reactivity.

5.3 Mucosal Atopy

The impact of mucosal atopy investigated in the experimental skin irritancy model is not as clear-cut as the impact of its dermal counterpart, AD. Greater responses to graded SLS solutions were observed in AD patients and in patients with mucosal atopy without a history of AD, compared with nonatopic controls (Nassif et al. 1994). In each atopic group, the effective SLS concentration causing irritation in 50% or more of the subjects (ED_{50}) was significantly lower compared with the controls. In a small study on a use test with a dishwashing liquid, persons with a history of mucosal atopy were not among the hyperreactors (Klein et al. 1992).

5.4 Prior Exposure to Irritants (Adaptation, Hardening)

In guinea pig skin, eight applications of detergents induced an increasing irritation (McOsker and Beck 1967). After 30 applications, however, there was a complete accommodation. The same phenomenon has been identified in multiple repeated exposures on humans (Tupker et al. 1989a) (see Fig. 2). In several healthy subjects, clinical changes were accompanied or preceded by a downgrade curve in the TEWL time course, after an initial TEWL increase. A similar TEWL time course was observed on skin sites showing no or only mild clinical signs (Tupker et al. 1989a).

TEWL decrease after initial increase was also noted in a 21-day repeated use test (Klein et al. 1992).

The individual eczema risk was assessed in a 2-week predictive washing test using SLS and a synthetic detergent solution in three groups, divided according to atopy score (Gehring et al. 1998). In the group with the lowest atopy score, 1 out of 16 subjects discontinued the SLS washings due to severe inflammatory reactions, whereas six subjects showed hardening. On the contrary, in the group with the highest atopy score ("atopy very likely" but no history of AD), 5 out of 10 subjects had to quit SLS washing. The remaining five volunteers tolerated SLS washing over the entire period without any clinical reaction or hardening.

More recently, it has been shown that there was hyporeactivity toward a challenge with a onetime SLS patch at sites already repeatedly exposed to SLS after 6 weeks and, to a lesser degree, after 3 and 9 weeks (Heinemann et al. 2005). This was accompanied with a significant increase in the amount of stratum corneum ceramides (ceramides 1, 2, 3, 5, 6, and 7) after 3 weeks and cholesterol after 3, 6, and 9 weeks. Particularly, ceramide 1 was upregulated after 3 weeks, followed by a decline.

Decreasing and increasing TEWL values were noticed in the third week of repeated 3-week SLS exposures in an investigation studying the influence of cytokines on the chronic skin irritation response (de Jongh et al. 2006). In contrast, erythema values continued increasing over the 3-week exposure period.

Interindividual variations in TEWL time course after a onetime patch test were demonstrated, which were inversely correlated with the responses after repeated occlusive exposures ($R = -0.61$) (Tupker et al. 1990b). TEWL increases observed in the days after patch removal were associated with higher TEWL values in the repeated test model, indicating the importance of hardening in a functional way.

Hardening of the skin may be an important factor in predictive testing on subjects with a different occupational background. For this reason, the skin of a cleaner may be less susceptible than that of an office worker. The specificity of hardening is not yet exactly known. If the specificity of hardening is high, one must be cautious of this phenomenon in predictive irritancy tests comparing different chemicals.

The traditional statement on the pathogenesis of chronic ICD, namely, that its development depends on the balance between the sum of all extrinsic influences on the one hand and intrinsic constitutional factors regulating the reparative capacity of

the skin on the other hand, has been elaborated into a broader hypothetical view (Elias et al. 1999). In this scenario, the epidermis is nowadays considered as a highly active site of lipid synthesis that is under direct control of alterations in barrier status (Elias et al. 1999; Willis 2002). Barrier injury, regardless of what type (detergents, solvents, tape stripping), evokes a recovery or adaptive response that leads to normalization of barrier function within hours to days. Perturbation of the ionic gradient in the epidermis and various cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and growth factors are involved in this process (Elias et al. 1999). This barrier-initiated cascade of cytokines is seen as a normal response, always accompanying the repair process after barrier disruption. In normal skin, chronic exposure of the epidermis to damaging insults will result in a compensatory hyperplastic state of the epidermis, also called adaptation or hardening. However, in AD, exposure to these damaging insults hypothetically induces recruitment and stimulation of inflammatory cell types in the epidermis, so that previously uninvolved skin sites turn into involved sites (Elias et al. 1999).

Thus, when the question is adapt or not adapt, AD patients may be grouped in the latter category, as is the case for some other subgroups of persons with a susceptible skin. Hopefully, future studies will clarify which (genetic) factors are involved in the process of adaptation and in factors involved in the hyperreactivity of AD skin (see Table 1).

Table 1 Factors probably involved in hyperreactivity to irritants in subjects with normal skin, susceptible skin, and atopic dermatitis

	Normal skin	Susceptible skin	Atopic dermatitis
Barrier function impairment (TEWL)	=	↑/=	↑
Filaggrin mutations	+/–	+/–	++[a]
Filaggrin phenotype downregulation	–	–	+
Mediators (TNF-α)	–	+	?
SC lipids			Ceramides 1/4 ↓
			Ceramide 7 ↑
Concomitant dermatitis elsewhere		+	++

[a]Higher prevalence in atopic dermatitis

5.5 Genetic Factors

5.5.1 Filaggrin Null Mutations and Barrier Function

Profilaggrin is a large polypeptide located in the keratohyalin F granules in the granular layer of the epidermis. During formation of the cornified envelope, multiple copies of the functional filaggrin (FLG) peptide units are formed (O'Regan et al. 2008). FLG binds to the keratin cytoskeleton, resulting in a flattened squame aligned parallel to the outer surface. Subsequently, the FLG peptide is degraded into a pool of hydrophilic amino acids, known as the natural moisturizing factor (NMF). Several loss-of-function FLG mutations were detected of which two mutations have a rather high (10%) prevalence in European populations (R501X and 2282del4). These mutations were found in about one-third of the AD population.

In a recent study on unselected volunteers, 16 carriers of a FLG mutation have been identified and compared with 23 subjects with the wild-type FLG gene (Kezic et al. 2008). The prevalence of past or present AD was higher in the carriers. Furthermore, the level of NMF was lower, and the TEWL of uninvolved skin was higher in carriers compared with noncarriers.

These findings have been corroborated by Jungerstedt et al. who have demonstrated that TEWL in a AD group with the FLG null mutation was a higher than in healthy controls with no null mutation (Jungerstedt et al. 2010). Besides the higher TEWL value, lower values for skin hydration were found in the AD group with the FLG mutant compared with the AD group with the FLG wild type and controls. The same phenomenon held true for skin pH, which was highest in the AD-FLG mutant group and lowest in the healthy FLG wild-type group, consistent with the putative lack of acid NMF in the former group.

In a Japanese study, four recently discovered FLG mutations (p.Ser2554X, c.3321del, p.Ser2889X, p.Ser3296X) have been investigated in 12 AD patients with an FLG mutation, 12 AD patients without mutations, and 12 healthy controls without mutations (Nemoto-Hasebe et al. 2009). It appeared that TEWL on uninvolved sites was higher in the AD patients *without* the mutation than in the AD patients with the mutation, which in its turn had higher values than the controls. A similar ranking order was found for SC thickness but not for skin hydration (AD with mutations had lower values than AD without mutations).

In a French study on AD patients, no influence of the "European" FLG mutations (R501X and 2282del4) could be demonstrated, neither on the AD severity nor on the TEWL value of noninvolved skin (Hubiche et al. 2007).

From these studies, it becomes clear that AD is associated with barrier function impairment, irrespective of the FLG gene status. Therefore, when delineating a subgroup with FLG null mutations, the remaining subgroup(s) without these mutations should have barrier defects due to other structural molecules or still undetected FLG mutations.

5.5.2 Mediators of Inflammation

The influence of a genetic marker on irritant susceptibility has been investigated (Allen et al. 2000). Visual irritant thresholds were determined using graded concentrations of SLS and benzalkonium chloride in a large group of nonatopics. Transition polymorphism has been identified in the TNF-α gene. Individuals carrying a haplotype that includes the A allele are high secretors of TNF-α, which is a key mediator in the pathogenesis of ICD. In the low irritant threshold groups of both SLS and benzalkonium chloride, a significantly increased number of persons having the A allele has been found. This study offers the first description of a nonbarrier-related marker of susceptibility in nonatopics.

In response to the SC damage by irritants, IL-1α, TNF-α, and other proinflammatory cytokines are released, resulting in an inflammatory reaction (in: De Jongh et al. 2006). To counteract these processes, keratinocytes also produce anti-inflammatory cytokines, such as IL-1 receptor antagonist (IL-1RA) and IL-10. However, this hypothetical view could not be confirmed in a study using a onetime patch and repeated SLS exposures (De Jongh et al. 2006). IL-1α decreased, and IL-1RA and IL-8 increased after repeated exposures. Positive correlations between baseline concentrations of IL-1RA and IL-8 on the one hand and TEWL and erythema increases after the 24-h patch test on the other hand were noted. Therefore, baseline IL-1RA and IL-8 levels may be indicators of greater skin reactivity after single exposure to SLS. However, no correlations could be observed between these cytokines and scores after repeated exposures. Regrettably, only a limited number of mediators were tested in this study, and the contribution of delta values for the cytokines on the skin responses was not investigated. The divergent influence of baseline cytokine levels between the single patch and repeated test may be explained by the adaptation observed in some participants, but these participants were not clearly indicated with respect to cytokine time course. Adaptation is a highly dynamic process probably involving a multitude of (partly known) mediators, each of which might show a time-dependent pattern.

A special feature of AD is the so-called T-helper-2 polarization, characterized by the predominance of IL-4, IL-5, and IL-13. In a study on 39 healthy subjects and 30 AD patients, three subjects with an FLG null mutation were identified in the AD group and two subjects in the healthy group (Howell et al. 2007). The FLG gene expression of the AD patients *without* a FLG null mutation was lower than that of healthy controls *with* the mutation but higher than that of AD patients with the mutation. Lesional skin exhibited higher values than nonlesional skin. In in vitro experiments, it was demonstrated that FLG expression was downregulated in the presence of IL-4 and IL-13, whereas IFN-γ upregulated this expression. It is concluded that the FLG deficiency in AD is acquired rather than constitutive and that local inflammation by T-helper-2 cytokines can decrease FLG gene expression in patients without the null mutation (Howell et al. 2007).

The strength of response to irritants is not only influenced by the levels of cytokines, such as TNF-α and IL-1, but also by the individual's ability to quench free radicals, levels of antioxidant enzymes, and the ability to form protective heat shock proteins. It has been hypothesized that the above-mentioned mechanisms may all be under genetic control, which thus determine the variability in responsiveness to irritants (Willis 2002).

5.5.3 Stratum Corneum Lipids

The amounts of total SC lipids are lower in AD compared with that of healthy skin (Imokawa et al. 1991). Among the ceramides, ceramide 1 is significantly reduced in both lesional and nonlesional AD skin. Ceramides are thought to be a key factor in the formation of the lipid bilayers between the corneocytes, resulting in water-holding function. After a 3-week repeated SLS exposure in healthy subjects, an increase in total lipid content of SC has been demonstrated (Heinemann et al. 2005). This increase was particularly true for ceramide 1. In a recent study, the dynamics of SC lipid metabolism have been analyzed in AD patients and healthy controls, in dependence of FLG genotype (Jungerstedt et al. 2010). AD patients with and without the FLG mutation had lower ceramide 4 levels and higher ceramide 7 levels than control subjects with and without these mutations. It was concluded that the exact phenotypic characteristics of FLG mutations with respect to the SC barrier are not fully understood and that the differences in ceramide subclasses may be related to AD per se and not representing a FLG-related feature.

In studying intrinsic, personal characteristics that may determine increased reactivity to irritants, AD has been shown a significant factor, both in the laboratory and in the occupational setting. However, it is not yet known what makes the AD skin unique in its reactivity. New findings point toward the role of FLG null mutations in lowering the barrier function in a subgroup of AD patients. Furthermore, characteristic SC lipid patterns have been identified in AD, irrespective of FLG status.

6 Predictive Testing in the Occupational Setting

In the past decade, several field studies have been conducted in which the incidence or prevalence of hand dermatitis has been investigated in a cohort of trainees in high-risk occupations. In these prospective studies, the aim was to examine the impact of one or more potential risk factors on the incidence of hand dermatitis. Important risk factors were history of AD, history of hand dermatitis, history of metal dermatitis, degree of chemical and mechanical exposure and insufficient recovery time during the work (Nilsson and Bäck 1986; Smit et al. 1994; Berndt et al. 2000; John et al. 2000). In some studies, the factor of history of AD has not been identified as a risk factor, probably because of selection bias (Smit et al. 1994; Coenraads and Pinnagoda 1985; Goh and Gan 1994; Smith et al. 2002). Apparently, only those persons having no history of dermatitis had chosen these high-risk occupations or remained in these occupations ("healthy worker effect"). Dry skin and mucosal atopy were risk factors in a study on hairdressers and nurses (Smit et al. 1994). Possibly, these high-risk persons belong to the group which may be regarded as having an "atopic skin diathesis," a term coined by Lammintausta and Kalimo (1981). Atopic skin diathesis was denoted as the presence of dry skin and low itch threshold for two of three irritants (sweat, dust, and rough materials), in combination with white dermographism and facial pallor/infraorbital darkening. In their cross-sectional study on "wet" hospital work, they found a strong influence of a history of AD but a very weak influence of mucosal atopy (Lammintausta and Kalimo 1981). However, past or present mucosal atopy in combination with atopic skin diathesis was proven to increase the risk of hand dermatitis (Lammintausta and Kalimo 1981). Diepgen has constructed an "atopy score" (Diepgen 1991), based on the criteria for AD by Hanifin and Rajka (1980), to better assess the likelihood of developing dermatitis in high-risk environments. In some field studies, however, the value of this atopy score was less contributive than a history of AD (John et al. 2000; Berndt et al. 1999a). In a

large-scale investigation on car industry workers, the following independent factors could predict the occurrence of hand dermatitis to a large extent: history of AD, history of hand dermatitis, wet work more than 3 h per day, and dyshidrosis (T.L. Diepgen, 2002, personal communication). For hairdressers, predictive factors were atopic skin diathesis, wet work more than 4 h per day, and exposure to permanent wave liquids (T.L. Diepgen, 2002, personal communication). In a recent case–control study, it was determined whether FLG polymorphism influenced susceptibility to occupational ICD (De Jongh et al. 2008). The prevalence of dermatitis which had started during their training was nonsignificantly higher in carriers (57%) of the FLG null alleles, compared with noncarriers (41%). In contrast, carriers reported significantly more frequently (43%) dermatitis on the hands before the start of their training than noncarriers (10%). A large Danish population study showed a higher prevalence of hand dermatitis among AD patients with FLG null mutations as compared with AD patients without FLG null mutations and non-atopic persons with or without FLG mutations (Thyssen et al. 2010). Therefore, determination of FLG null mutation may have an additional predictive value in persons with a history of AD.

In contrast to the findings in experimental skin irritancy studies, prospective field studies could not demonstrate the importance of preexposure barrier function as a risk factor for hand dermatitis (John et al. 2000; Smit et al. 1994; Goh and Gan 1994; Berndt et al. 1999b; Schmid et al. 2005), with the exception of a study by Coenraads and Pinnagoda (Coenraads and Pinnagoda 1985). In this relatively small study, the incidence of hand dermatitis was much lower than in the field studies conducted afterward. A possible reason for the elevated preexposure TEWL in the persons, who turned out to be cases later on, is the fact that these persons might already have been exposed during previous occupations. In other studies, increased TEWL values were observed preceding the occurrence of hand dermatitis (John et al. 2000; Goh and Gan 1994).

The use of provocation tests as a tool for the prediction of risk for hand dermatitis has recently been investigated, since preexposure barrier function had been proven to be of no value for this purpose (Smith et al. 2002; Berndt et al. 1999a). Tests with SLS or dimethyl sulfoxide (DMSO) were not able to predict hand dermatitis, in contrast to those with NaOH (Berndt et al. 1999a). The combination of the provocation values of DMSO and NaOH and skin moisture had a high sensitivity (94%) but a low specificity (24%) (Berndt et al. 1999a). In a small study by Smith et al., 9 out of 24 apprentice hairdressers developed hand dermatitis (Smith et al. 2002). In these cases, the preexposure irritation threshold tended to be lower than in noncases. Unfortunately, a 4-h onetime SLS test was performed, which was assessed visually, in this investigation in which 16 out of the initial group of 42 persons were lost to follow up for "administrative reasons" (Smith et al. 2002).

The point prevalence of ICD was higher during the first observation period (Berndt et al. 2000; Goh and Gan 1994; Funke et al. 2001). This is probably due to the phenomenon of hardening developing during the later periods. Another possibility is a heavier exposure to irritants in trainees during their first work period. Self-reported skin symptoms improved during their training in 12 out of 104 apprentice nurses, apparently through hardening (Schmid et al. 2005). However, 19 other apprentices reported symptoms for the first time halfway through their training despite the fact that those persons had reduced the frequency of hand washing and improved the use of skin care products.

Field studies on workers in high-risk occupations have shown that prework barrier function is not a valid predictor of the risk of hand dermatitis, in contrast to preexposure barrier function in experimental irritancy models. In these laboratory models, irritants are exposed for a relatively short-time period on the skin. In daily practice, however, the result of repeated exposures to damaging influences of various kinds is a complex interrelationship of different (partly unknown) factors, such as severity of chemical and mechanical insults, recovery time between exposures, seasonal influences, barrier function, history of AD, ability to develop adaptation, and other factors that are probably under

genetic control. Determining who is at risk probably demands a combination of anamnestic data, well-performed irritancy tests, and genetic tests (in the future). Predictive irritancy testing by means of one or more standard irritant(s) may be useful in the occupational setting. This probably requires that several irritants be tested since, in reality, there is exposure to different kinds of irritating factors (Berndt et al. 1999b). It is a challenge to find out which testing method is most appropriate for a particular occupation. Once it is decided which method is most appropriate, officially agreed guidelines for that method should be developed.

In view of the complex interrelationship of the above-mentioned factors in daily practice, multiple repeated exposure models should be used as a tool to identify persons at risk for hand dermatitis in field studies, because only in this way can essential factors, such as the ability to develop adaptation, be tested. The disadvantage of such a repeated model, however, is its lower practicability in the field. A solution for this problem might be to perform TEWL measurements before and after some weeks of relevant exposure to the types of irritants involved in the occupation concerned, as field studies have demonstrated TEWL increases in workers who later turned out to be cases of hand dermatitis (John et al. 2000; Goh and Gan 1994).

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