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Combined effects of allergens and irritants

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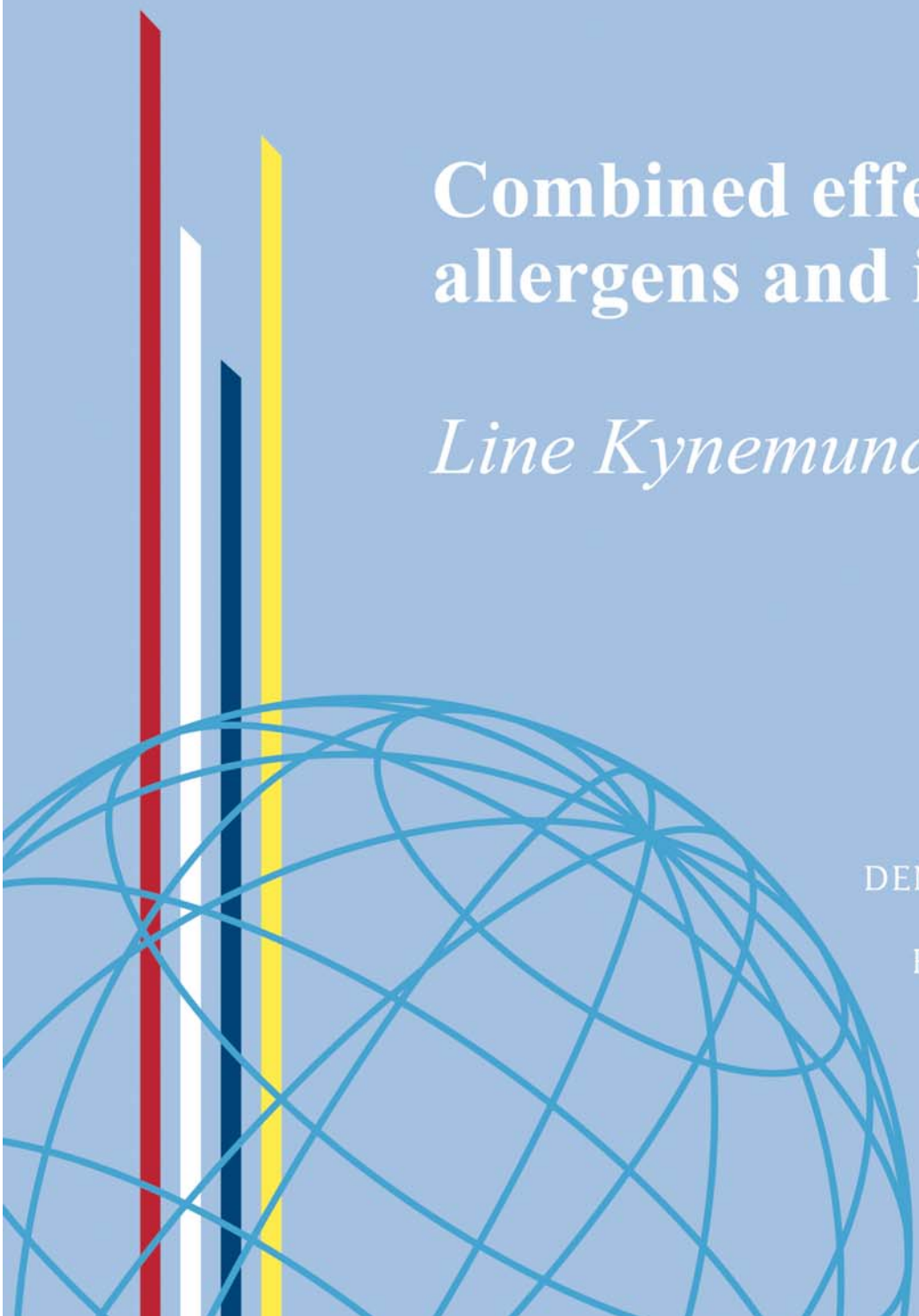

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Combined effects of allergens and irritants

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LIST OF ABBREVIATIONS

ACD	Allergic contact dermatitis
CD	Contact dermatitis
D	Day
DNCB	2,4-dinitrochlorobenzene
Eth	Ethanol
ICD	Irritant contact dermatitis
MDBGN	Methyldibromo glutaronitrile
OR	Odds Ratio
Pet	Petrolatum
Ppm	Parts per million
ROAT	Repeated open application test
SC	Stratum Corneum
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers
SLS	Sodium Lauryl Sulfate
TEWL	Transepidermal waterloss
TNF-α	Tumor necrosis factor alpha
UV	Ultraviolet light

This thesis is based on the following publications:

I. Pedersen LK, Haslund P, Duus Johansen, J, Held E, Volund A, Agner T. Influence of a detergent on skin response to Methyldibromoglutaronitrile in sensitised individuals. Contact Dermatitis 2004; 50: 1–5.

II. Pedersen LK, Agner T, Held E, Duus Johansen J. Methyldibromoglutaronitrile in leave-on products elicits contact allergy at low concentration. Br J Dermatol 2004; 151: 817–822.

III. Pedersen LK, Duus Johansen, J, Held E, Agner T. Short-term effects of alcohol-based disinfectant and detergent on skin irritation. Contact Dermatitis 2005; 52: 82–87.

IV. Pedersen LK, Duus Johansen J, Held E, Agner T. Less skin irritation from alcohol-based disinfectant than from detergent used for hand disinfection. Br J Dermatol In press.

In the text these papers are referred to by their respective roman numerals.

CONTENTS

PREFACE

This Ph.D.-thesis was prepared at the National Allergy Research Centre, Department of Dermatology, Gentofte Hospital, Hellerup, Denmark in the period 2002 to 2005. I wish to thank all those who helped and contributed to this work.

In particular, I would like to thank my main supervisor, dr.med. Tove Agner, who introduced me to the field of irritant contact dermatitis and experimental dermatological research. Not only has Tove Agner´s never-failing support and enthusiasm been invaluable to me over the years, but also her always-constructive criticism, which has motivated me throughout my study time.

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Gentofte, March 2005
Line Kynemund Pedersen

INTRODUCTION

Contact dermatitis (CD) is a frequent disease in the industrialised world. It is mainly caused by environmental exposures. CD can be divided into irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). CD represents a clinical reaction, initiated through either contact with allergens or through contact with skin irritants. Typically, in both types of CD, the skin is dominated by T lymphocytes; however, clinically, ACD and ICD are impossible to distinguish.

More than 15% of the Danish population has contact allergy to one or more chemical substances (1, 2). Once CD has been elicited the risk of chronicity is high, and, in many cases, this may be elicited by continual exposure to irritants and relevant allergens in the work place or in the home. The damaging effect to the skin caused by irritants depends on both the concentration of the irritant and on exposure time. A dose-response relationship exists for allergens, and elicitation of allergic contact dermatitis (ACD) depends on the dose of the allergen. The presence of more irritants together or irritants and allergens in combination may, however, potentially influence skin response to irritants, and for allergens the elicitation thresholds may be influenced considerably. Since CD is the most frequently reported occupational disease, society has an interest in its prevention. Knowledge about how combined exposures influence skin reactivity is important for prevention of CD.

IRRITANT CONTACT DERMATITIS (ICD)

ICD is believed to be commoner than ACD (3-6). ICD is elicited when an overbalance occurs of irritant factors in relation to defence and repairing capacity of the skin. Several different types of clinical ICD exist (7). Acute ICD results from a single exposure to an irritant provided the amount of irritant is sufficient and the molecule is potent enough. It is often accidental and recognised early (8). The clinically commonest form of ICD is chronic ICD. When repeated exposure to the same stimulus or to a combination of stimuli reaches a critical level it results clinically in ICD (9). In chronic ICD the barrier function is disrupted and is associated with increased epidermal turnover leading to histological hyperproliferation and transient hyperkeratosis, and clinically leading to lichenification, dryness and fissuring (9).

Exogenous variables which influence susceptibility to irritants include type of irritant (chemical structure, pH), intensity of exposure to irritants and mechanical factors. Seasonal variation and ambient humidity also play a role in skin susceptibility, and increased skin susceptibility has been reported during winter compared to summer (9), probably due to a decreased hydration state of the epidermis (10,11). An important endogenous factor that increases individual susceptibility is atopic dermatitis (12). In an individual, susceptibility to irritants may change over lifetime. Within the age range of 18–50 years, no significant influence of age on skin susceptibility should be expected, whereas in older people, less skin reactivity to irritants is reported (9). Increased susceptibility to SLS in the menstrual cycle on D1 as compared to D9 and D11 in healthy women has been shown (13); however, most investigations have found no difference between males and females in skin susceptibility (14, 15, 16). Regional differences in susceptibility to irritants between different anatomical regions have been discussed, and face and jaw angle was reported as high-absorbing skin sites (17). Variability in responsiveness to irritants is also influenced by genetic factors (18). Reactivity to one irritant does not necessarily predict the susceptibility to others (12). The inflammatory reaction to irritants is highly complex, and immunological processes are also important in initiation of ICD.

WET WORK AS A RISK FACTOR FOR ICD

A generally accepted German definition of "wet work" is: "Skin exposed to liquids longer than 2 h per day, or very frequent washing of the hands (>20 times/day or fewer if the cleaning procedure is more aggressive)" (19). Relevant wet work occupations are e.g. health-care work (hospital employees), cleaning, food handling and hairdressing. Wet work is a major risk factor for developing hand eczema, and at least doubles the risk compared with dry office work (20). Besides wet work in the workplace, women are often exposed to wet work in the home, which also increases the risk of developing ICD (21). Malten described the pathogenesis of chronic ICD relevant for wet work (22).

DIFFERENT EFFECTS OF IRRITANTS – SLS AND ALCOHOL-BASED DISINFECTANT AS MODEL IRRITANTS

The model irritant sodium lauryl sulphate (SLS), a detergent (surfactant), is used to induce experimental ICD. SLS disrupts the skin barrier, and the most precise measure of skin barrier defect due to SLS is an increase in TEWL (23). Alcohol-based solutions are often used for hand disinfection, and alcohols are used as preservatives in cosmetic products (9). Clinically important alcohols added in hand-disinfectants are ethanol (eth), 1-Propanol and 2-Propanol. Compared to the barrier-disrupting agent SLS, these alcohols primarily generate an inflammatory response in the skin, causing skin irritation with only little effect on the skin barrier. Laser-Doppler flowmetry for measurement of skin blood flow, colourimetry for measurement of skin redness and ultrasound for measurement of oedema formation can be used to quantify the inflammatory response (24).

CHANGES IN SKIN REACTIVITY: HARDENING

In the individual, the response to irritant stimuli depends on the skin barrier function and the inflammatory reactivity of the skin, and in the case of chronic ICD, the skin's regeneration ability or ability to develop hardening. Hyporeactivity or "hardening" is defined as depressed skin reactivity weeks after exposure to an irritant or an allergic stimulus (9). Development of hardening may be specific to the irritant or allergen tested, and may be due to an increased thickness of stratum corneum (SC) (25) and changes in the composition of SC lipids (26). Another mechanism may be down-regulation of inflammation (27), indicating a changed immunological response due to a specific T-cell memory function in the skin. Decreased skin reactivity has been reviewed (28). Widmer et al. observed decreased skin reactivity to SLS when applied after 6 weeks to a test site with previous ICD from SLS, indicating a hardening effect of SLS (26). Hindsén & Bruze observed decreased skin reactivity to nickel when applied after one month to a test site with previous ICD from SLS (29). Additionally, no increased skin reactivity on skin with previous ACD from another allergen was found, indicating an allergen-specific memory function in the skin (29). The current knowledge about hardening is patchy, and more studies are required.

ALLERGIC CONTACT DERMATITIS (ACD)

ACD is a cell-mediated type IV immunological reaction to one or more specific allergen(s) in an individual already sensitised. Common cases of ACD come from exposure to allergens such as metals (e.g. nickel, Ni), fragrances and preservatives (e.g. methyldibromo glutaronitrile, MDBGN). In 1939, Landsteiner and co-workers (30) performed human sensitisation experiments and established an individual variation in susceptibility to contact sensitisation. They also showed that individuals who were highly susceptible to sensitisation with one chemical showed little or no susceptibility to sensitisation with others. The genetic component in ACD was recently studied in a twin population, and it was concluded that environmental factors were of major importance in relation to nickel allergy (30).

IMMUNOLOGICAL MECHANISMS IN ALLERGIC CONTACT DERMATITIS – "THE DANGER MODEL"

It has been suggested that a "danger signal", interpreted as initiation of an immunological process, is of importance for sensitisation and elicitation of ACD, and the concept that an irritant or an irrelevant hapten is necessary to provide this danger signal has been suggested (31, 32). Grabbe et al. (33) proposed that antigen-specific cell activation and a non-antigen-specific proinflammatory signal are necessary to induce contact hypersensitivity (CHS) and that both actions might be provoked by application of a sufficiently high dose of hapten (33). In an experimental study, simultaneous application of a relevant allergen, e.g. an allergen to which the person is sensitised, and an irrelevant allergen, e.g. an allergen to which the person is not sensitised, caused ACD, while single application of each compound did not. It was shown that the presence of an irrelevant hapten contributes to elicitation of ACD (33). This study was performed on mice; performing a similar study in humans would be hazardous, because it may cause contact sensitisation.

It is documented that the proinflammatory cytokine TNF-α, plays a key role in ACD, in both sensitisation and elicitation phases, as well as in ICD. Due to additional irritant effects, some allergens, e.g. 2, 4-dinitrochlorobenzene (DNCB), influence the immune response in the epidermis by upregulation of TNF-α (34, 35, 36). This indicates that the danger signal in ACD is cytokine release, e.g. TNF-α from keratinocytes (32).

Two studies on the genetic influence on the susceptibility to ACD and ICD (37,38) proposed that a polymorphism in the gene encoding for TNF-α (TNFA-308*2) was involved in both skin irritation and sensitisation, reflecting common pathogenetic pathways of these types of dermatitis. This finding may point to a genetic basis of the "danger model".

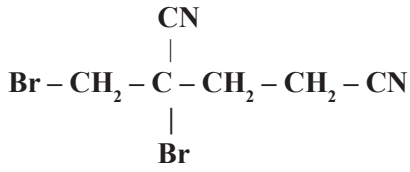
It has been discussed whether individuals with a low threshold to irritants, such as SLS may also be more easily sensitised than normal individuals, and also that these individuals are more liable to develop ACD (39). A recent study indicates that individuals with a positive colophony patch test have a lower irritant threshold, and thus a greater susceptibility to skin irritation, than normal individuals (40). Another study investigated skin reactivity to irritants in relation to sensitisation and/or elicitation of DNCB allergy (41), and there, no association between reaction to DNCB and irritant threshold (reaction to SLS) was found when evaluated by visual scoring, while ultrasound measurements indicated a possible association. The questions are still open as to whether an association between low threshold values for elicitation of ACD and increased skin susceptibility to irritants exists.

DOSE-RESPONSE RELATIONSHIP FOR ALLERGENS

For both weak (e.g. parabens and lanoline) (42, 43) and strong contact sensitisers (e.g. DNCB, chloroathranol) (41, 44, 45) the concentration of allergen, dose per unit area, is crucial for sensitisation of non-sensitised persons and possibly also for elicitation of ACD in already sensitised persons. Dose-response dependency may be changed by addition of an irritant (44, 46, 47). Endogenous factors e.g. immunological status (Langerhans' cell density and release of cytokines) and previous contact eczema also play an important role in influencing the propensity of already sensitised persons to elicit a reaction.

METHYLDIBROMO GLUTARONITRILE – AS A MODEL ALLERGEN

In the mid 1980s, a new compound for preserving cosmetic products was introduced in Europe. The compound, Euxyl K 400 (Schülke & Mayr, Hamburg) was a combination of the two preservatives methyldibromo glutaronitrile (MDBGN) and 2-phenoxyethanol at a ratio of 1:4. The combination



1,2-Dibromo-2,4dicyanobutane
CAS 35691-65-7

Fig. 1. Chemical structure of Methyldibromo glutaronitrile.

was very effective against a broad spectrum of bacteria and fungi in relatively low use-concentrations; consequently, Euxyl K 400 became increasingly popular. The preservative has been used in a wide range of cosmetic products such as shampoos, soaps (rinse-off products) and moisturisers (leave-on products), and also in cleaning products and various industrial products.

The main sensitising compound is MDBGN (Fig. 1) and cases of ACD from 2-phenoxyethanol are only rarely seen (48, 49, 50).

The first reports of allergic contact dermatitis to MDBGN were published in 1983 due to exposure to a paste glue formation (51). Later, cases of ACD from MDBGN in cosmetic products were reported (52).

For many years MDBGN has been permitted at a maximum concentration of 0.1% (Euxyl K 400 0.5%) in both rinse-off products and leave-on cosmetic products. However, after the documentation of an epidemic of contact allergy to MDBGN (53), the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) advisory to the EU-Commission recommended that the use of MDBGN in leave-on cosmetic products should be discontinued until a safe use concentration could be established (54), while the use of MDBGN in rinse-off products could remain unchanged at a maximum of 0.1%. Thus MDBGN is still used in much liquid soap. Clinical and experimental evidence of its significance as an allergen in rinse-off products has been published (55).

The clinical case of ACD to MDBGN is more severe than those usually seen for cosmetic dermatitis; a predominant characteristic is hand eczema (56, 57). MDBGN was used in the current investigations as a model allergen, since it is a frequent cause of contact allergic reactions from both moisturisers and liquid soaps, where it is often present in combination with detergents.

COMBINED EFFECTS

Development of CD in real life is often due to simultaneous exposure to allergens and irritants. Typical examples of combined exposure are consumer products, cosmetics or cleansing products, where numerous contact allergens are often present in combination with detergents. Combined exposure to two allergens or to irritants and allergens in combination may markedly change the threshold value for elicitation of ACD in already sensitised individuals. The combined exposure to two irritants may result in a different skin response than exposure to each irritant individually. Mechanisms for changed responses can be immunological effects or enhanced penetration. Several types of interaction exist: 1) an additive response where the skin response is that expected from adding the responses to each of the substances when applied separately; 2) a synergistic response where the skin response is greater than that expected from adding the separate responses; and 3) an antagonistic response "quenching" where the skin response is less than that expected from adding the separate responses (31). In the following, focus is on possible additive and synergistic responses to combined exposures to irritants and allergens.

COMBINED EFFECTS OF TWO ALLERGENS

Elicitation of ACD depends on the concentration and availability of the applied allergen. The combined exposure to several contact allergens potentially influence allergenicity and availability of the substances, and mechanisms for a changed response are likely to be either because of an immunological effect or due to changed availability (31). Knowledge about how combined exposures influence skin reactivity is important for prevention of ACD. Both additive and synergistic responses have been reported. In a human study by McLelland & Shuster (58), the response to two different allergens applied simultaneous was evaluated. Different allergens were used, all subjects were allergic to both allergens, and the allergens were each applied in sub-threshold doses. It was concluded that the threshold for a response to one allergen was lowered by the presence of another and that the interaction was an additive response. Combined effects of two allergens were also studied by Johansen et al. (59) by comparing two groups of patients; one group consisting of patients with two allergies and a control group with only one allergy. Both groups were exposed to two allergens (perfume ingredients), alone and in combination. In individuals sensitised to both allergens, the response was augmented by about a factor 4 as compared to the allergens tested separately, while in control subjects, no increased response was observed for the combined allergens. The increase in skin response after combination of two allergens was reported to be synergistic (59). Differences in statistical evaluation of data may well explain the different conclusions concerning additive or synergistic effects of combination. Nevertheless, both studies indicate a significant interaction between allergens when applied in combination.

COMBINATION OF AN ALLERGEN AND IRRITANT

In daily life, combined exposure to irritants and allergens is very common, since many consumer products such as liquid soaps and cleansing products often contain both allergens and irritants. Interaction may occur, either due to an irritant-induced upregulation of TNF-α, relevant for initiation of contact hypersensitivity, or due to an increased penetration of the allergen, facilitated by barrier disruption caused by the irritant. Detergents, in particular, harm the barrier (60, 61).

Simultaneous application of irritants and allergens has recently been reviewed (31). Kvorning & Svendsen (62) found that addition of a detergent solution to nickel chloride and potassium dichromate patch tests in patients allergic to these substances elicited allergic reactions that would otherwise have been negative. Kligman (63) performed a study in which he found that pre-treatment of the skin with 10% SLS 1 h before application of Ni magnified the response compared to single application of the allergen. Allenby & Basketter (64) reported that immersion of skin in SLS solution greatly enhanced patch test reactivity to nickel on the dorsal forearm, when compared with normal skin on the contralateral non-immersed forearm. Heydorn et al. (65) recently showed that a stronger response was elicited to the perfume allergen hydroxycitronellal when applied together with SLS than when applied alone. The real-life effect of combining the preservative MDBGN 1000 ppm with a detergent as in rinse-off cosmetic products was recently evaluated (55). It was shown that even with a short-term exposure only twice a day an allergic response was elicited in 1/3 of the sensitised individuals. In a study by McLelland et al. (46), patch tests with different standard allergens for 24 h followed by 24 h irritant patch testing (SLS or anthralin) were performed, and an increased, additive skin response was found, compared to application of the allergen alone. Agner et al. (66) studied the combined effects of nickel and SLS in a group of patients with nickel allergy. They found that the skin response to a combination of nickel and SLS was augmented by a factor 4 as compared to each of the substances applied separately, and using a logistic dose-response model for statistical analysis the response was found to be synergistic. This differs from the findings of McLelland et al. (46), who found an additive response, that may be due to a different design, simultaneous exposure of allergen and irritant. The interactive effect of al-

lergens and irritants in combination is supported by several studies. SLS or other detergents have been the preferred experimental irritants; this is highly relevant to real-life situations. However, conclusions made from studies with detergents cannot necessarily be generalised to other irritants.

COMBINED EFFECTS OF TWO IRRITANTS

Combined exposure to two irritants simultaneously and sequentially as repeated applications within the same individual often occurs in real-life in wet work occupations such as the health sector. It is generally accepted that detergents mainly affect skin barrier function, while other irritants primarily affect the inflammatory skin response. This different impact on the skin from different irritants makes the combined effect of simultaneous exposure to more than one irritant unpredictable, and the interaction of two irritants simultaneously may differ for the specific irritants combined.

Wigger-Alberti et al. (67) studied the skin response to SLS and toluene (an organic solvent) in a repeated irritation model (31). An increased skin response to combined exposure of SLS/toluene was found compared to each irritant individually, when evaluated by visual score, transepidermal water loss (TEWL), colourimetry and skin capacitance (68). The same authors also investigated the combined effect of SLS and n-propanol (69) using the same repeated irritation model; in this study, application of SLS and n-propanol did lead to increased skin irritation compared to the irritation caused by each substance alone. The irritant properties of different irritants may, however, differ greatly, and the conclusions cannot be generalised or predicted. In real-life wet work situations, alternating exposure to detergent and alcohol solutions often occurs. Further knowledge about interaction between detergents and alcohol is necessary to ensure optimal recommendations for skin disinfection/cleansing in wet work situations.

LIPID CONTENT AND SKIN BARRIER PENETRATION

The amount of lipids in the skin represents an important factor in susceptibility to irritation (27). Moisturisers used for treatment of ACD and ICD contain different amounts and sorts of lipids. The moisturisers are applied to repair the skin barrier which is disrupted in eczematised skin. Ceramides, cholesterol and free fatty acids play an important role in regulating the skin condition in the lipid layer in the SC. Moisturisers with a high lipid content have been shown to be more effective than those with a low lipid content in the treatment of ICD (70). However, the lipid content of the moisturiser has also been shown to influence skin response to exposure to irritants and allergens. Pre-treatment of normal skin with a high lipid content (70%) moisturiser has been shown to enhance the penetration of the allergen and thereby lower the threshold for elicitation of ACD (71). Induction of ICD is also shown to be influenced by the lipid content of a moisturiser, and high lipid moisturisers on normal skin have been shown to enhance skin reactivity to SLS (72).

HYPOTHESES AND AIMS OF THE THESIS

MAIN HYPOTHESES

1. The threshold value for a reaction to the preservative MDBGN is decreased and skin reactivity to this agent is increased by simultaneous application of SLS.
2. Repeated exposure to a single local irritating agent causes less inflammatory reaction than repeated exposure to two chemically different irritants.
3. The threshold value for elicitation of ACD from MDBGN is higher in a moisturiser with a high lipid content than in a moisturiser with a low lipid content.

OTHER HYPOTHESES

1. Skin disinfection with detergent is more skin barrier disrupting and generates more inflammation than skin disinfection with alcohol-based solution.
2. Hardening will develop on skin sites previously exposed to irritants.
3. A safe level of use for MDBGN is 50 or 100 ppm

THE SPECIFIC AIMS OF THE STUDIES WERE:

Study I

To evaluate the combined effect of the preservative MDBGN and sodium lauryl sulphate (SLS) on the elicitation response of ACD in MDBGN allergic individuals.

Study II

A) To evaluate if the lipid content of a moisturiser influences the risk for elicitation of MDBGN allergy in already sensitised individuals.

B) To evaluate if 50 or 100 ppm MDBGN in a moisturiser can be tolerated by MDBGN sensitised individuals.

Study III and IV

A) To test if repeated disinfection with detergent causes more skin irritation than repeated skin disinfection with alcohol-based disinfectant.
B) To test if alternate application of disinfectant/detergent causes more skin irritation than disinfectant and detergent applied alone.
C) To test if any of the irritants decrease skin reactivity 4 weeks after ended application (hardening effect).

METHODS

PATIENTS

Participants in study I and II were all eczema patients previously tested positive to MDBGN in the period 1999 to 2002 in routine diagnostic patch testing at the Department of Dermatology, Gentofte University Hospital. All patients had been patch tested with 0.3.% MDBGN in pet. and a +1 reaction was a minimum required for participation. If a positive patch test could not be reproduced during the trial, the subject was excluded. Further exclusion criteria were age below 18 years, pregnancy, UVA or UVB treatment, use of sun beds within the previous 2 weeks, immunosuppressive treatment, or other skin diseases and dermatitis on the test areas. Participants in study III and IV were healthy volunteers recruited by advertisement.

All participants provided written informed consent and the study was performed according to the Helsinki Declaration II. Approval was obtained from the local ethical committees.

ASSESSMENT OF SKIN REACTIONS

Visual scoring

Allergic patch test reactions were classified according to the International Contact Dermatitis Research Group (ICDRG) as +?, +, ++, +++, and IR (73). A positive patch test threshold value was the lowest patch test concentration to produce a positive reaction in an individual as a measure of the sensitivity to the allergen.

Readings of irritant patch test reactions were made on D2, D5, D7 and D11 and classified according to visual scoring for irritant reactions by Kligman and Frosch (74). The maximum possible score for this scoring system is 10.

Use-test reactions were evaluated by a scale developed by Johansen et al. (75). The scale is used for grading morphology of the use-test reactions; an overall clinical assessment of the test reaction can also be made using 5 points as cut-off value for a positive ROAT (75). Clinical assessments were made on day 2 (D2), D3, D7, D14, D21 and D28.

Bioengineering methods

Non-invasive bioengineering techniques are used for assessing allergic and irritant skin reactions. They are objective and able to detect and quantify skin reactions that are sometimes otherwise invisible to the naked eye. Transepidermal water-loss (TEWL) is passive diffusion of water through the stratum corneum (SC) and is, when sweating is kept to a minimum, an indicator of the integrity of the skin water permeability barrier. When the integrity is impaired, higher TEWL values are obtained. Evaporimetry is a highly sensitive and precise technique for evaluation of irritant skin reactions (23,76). The skin surface colour can be quantified using the standard tristimulus system suggested by the Commission International de l´Elairage (CIE) (Robertson 1997). Colour measurement using the Minolta Chromameter CR-200/CR-300 (Osaka, Japan) is based on illumination of the skin by xenon flash-light. The colour is expressed in a 3-dimensional coordinate system. Luminance (L*) expresses the brightness (integrated reflection of light from the surface), ranging from total black (low values) to pure white (high values). The a* and b* are the two colour coordinates: a* represents the colour range from green (-) to red (+), and b* the colour range from blue (-) to yellow (+). The true colour of the skin is expressed as an admixture of the a*, b* and L* values. The a* value is an indicator of the presence of haemoglobin, but it is also influenced by other chromophores (melanin, haemoglobin, bilirubin, carotene), and by structural conditions in the skin. A high a* value indicates increased inflammation. Erythema, as measured by Minolta Chromameter (colourimetry), has been demonstrated to correlate well with visual scoring in eczematous reactions caused by SLS (77). A positive correlation between increase in the a* colour coordinates and increased doses of SLS has been reported (78).

PATCH TEST

For both allergic and irritant reactions the Finn Chamber patch testing technique (Epitest Oy, Helsinki, Finland) was used, 8 mm Finn chambers were used for testing with

MDBGN and 12 mm Finn chambers were used for testing with SLS. 15 µl or 50 µl, respectively, of MDBGN or SLS was micropipetted onto filter paper discs of Finn Chambers (Epitest Oy, Helsinki, Finland) on Scanpor tape (Alpharma A/S, Vennesla, Norway) and mounted on the subject's upper arms. For patch test reactions to MDBGN, the patches were removed on day 2 (D2) by the subject and readings were done on D3 and D7. A commonly used MDBGN patch test concentration is 0.3% in pet. However, in some studies it was shown that this concentration dismisses clinically relevant cases of MDBGN allergy. The future recommended patch test concentration seems to be 0.5% (79).

The preferred MDBGN patch test vehicle is pet. However, it is difficult to dose pet. accurately and the solubility of MDBGN in pet. is limited. Therefore it was decided to use a 50:50 ethanol/water (Eth. /aq) vehicle.

SLS was used as a model irritant and guidelines on SLS exposure tests are available (80). Irritation was done as closed patch testing for 24 hours with a Finn Chamber ® containing an aqueous SLS-solution.

USE-TEST

Allergens

The ROAT (repeated open application test) is a frequently used method which simulates everyday use of dermatological cosmetic products. A test area of 3×3 cm, 5×5 cm, 5×10 cm or 10×10 cm is used; a smaller test area is not practical (55, 81).

The reactivity in ROAT depends on the concentration (dose) of allergen, the allergen, the region of application, e.g. the neck (Fig. 2), previous exposures, frequency of application, reactivity of the skin and the vehicle used. The number of days until a positive ROAT was initially shown to be 7 days as 80% of the patients reacted within this period (81, 82).

Irritants

The wash test and the ROAT are used in provoking irritant contact reactions (83). These tests simulate real-life conditions in their use. However, there are large interindividual variations and lack of standardisation when performing use-tests with irritants. This makes it difficult to compare the test methods.

One regime to perform the wash test is application of e.g. 3 ml liquid soap for 1 min twice daily for a week (2 applications daily). Different volumes have been suggested (84-88). ROAT can be done using e.g. 0.1 ml applied to an area of 5×5 cm twice daily without rubbing for one week (2 applications daily) (83).

In the studies of irritants, an intensified regime was carried out with 24 or 12 repeated applications daily for 2 days or 10 days with exposure to either detergent, alcohol-based solution or alternating detergent/alcohol-based solution as was used in study III and IV, respectively. Details of the respective products are given in study III and IV.

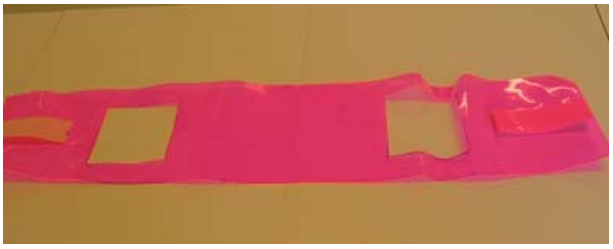


Fig. 2. Collar used for application of moisturiser in study II.

DISCUSSION OF EXPERIMENTAL STUDIES

STUDY I

In study I it was tested if the combined effect of MDBGN and SLS would influence elicitation of ACD from MDBGN.

Generally, combined exposures of allergens and irritants are important when determining safe-use levels of allergens. MDBGN is a relatively new, important allergen, primarily used in rinse-off products. Although the time of contact with the skin is shorter for a rinse-off product than a leave-on cosmetic product, the presence of detergents in soaps may alter the skin response significantly and make elicitation of an allergic response possible despite the short contact time.

Study I showed that an augmented skin response was found after concurrent application of MDBGN and SLS in a patch test. The skin response in sensitised individuals was augmented by a factor 6.4. Thus less MDBGN is needed to produce a response in combination with the detergent than MDBGN alone. This result is important for determining threshold values and risk assessment of contact allergens in consumer and industrial products, where allergens and irritants may occur in combination with detergents. The pathogenesis for interaction which caused the combined response is unclear, but increased penetration of the allergen due to irritant (SLS) induced skin barrier disruption is one likely explanation. The concept of the "danger" model with "danger" signals produced by irritancy may, however, also be in accordance with the result (31, 89, 90). In the present study, SLS was used as an irritant and it is also known that MDBGN at higher concentrations provides irritant effects. Irritants at sub-inflammatory levels can still augment elicitation reactions such as cytokine release and

keratinocytes. The interactive effect was additive rather than synergistic, as the effect of SLS on MDBGN response was constant (Figs. 3 and 4).

Only one relatively weak concentration of SLS was used together with low concentrations of allergen and with an exposure time of 24 h, compared to the 48 h normally used at patch-testing. The choice of exposure time and SLS concentration may have influenced the result. Thus another design using higher and different concentrations of SLS or longer exposure time may further clarify the type of combination effect.

The doses of SLS and MDBGN were chosen because they reflect daily life exposures. Other studies have investigated the combination of metals, e.g. chromium (Cr), Ni and detergents. Cr may be present in household products and thresholds for allergic reactivity to Cr have been studied in the presence of SLS (91). It was shown that the threshold for response was altered from 10 ppm to 1 ppm Cr in the presence of SLS, which is in the same order of magnitude as in the present study. In a recent study of nickel chloride ($NiCl_2$) and SLS in combination (66), the response was augmented by a factor 4 as compared to nickel alone. In a study of the combined effect of two fragrance ingredients, the response was augmented by a factor 4 also. The present study illustrates that the threshold value for elicitation of allergic reactions in MDBGN sensitised individuals is influenced by the presence of a detergent in low concentration. Results from this study and from previous studies on combined effects indicate that the augmentation of the skin response due to combination is within the range 4 to 10. This result is important and relevant for real-life exposure to many types of products such as rinse-off cosmetics. It shows that the presence of detergents in a product needs to be considered when assessing the risk for elicitation of ACD and in deciding cut-off values to protect the allergic individual.

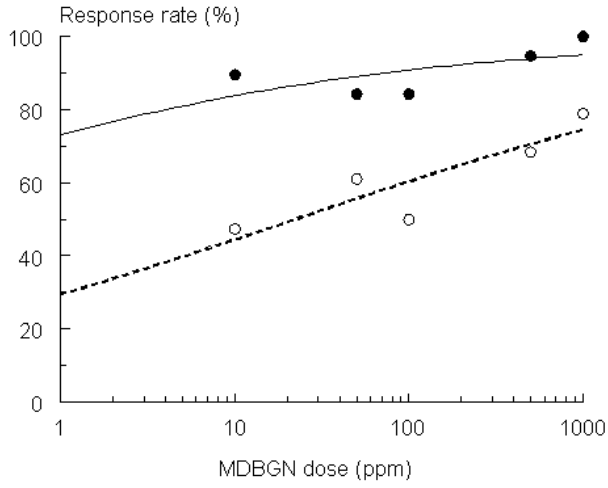


Fig. 3. Dose-response curve on D3 for clinical allergic response ≥?+ to Methyldibromo glutaronitrile alone and in combination with SLS. The black dots illustrate the dose-response curve for MDBGN+0.25% SLS. The white dots illustrate the dose-response curve for MDBGN alone. The response was augmented by a factor 6.4, expressed as an odds ratio, with confidence limits of 2.8 –14.6, p<0.0001.

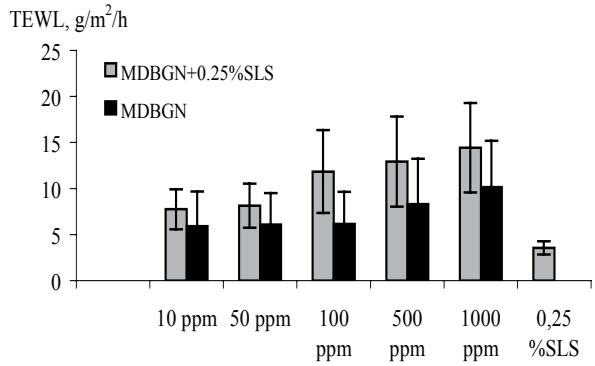


Fig. 4. Transepidermal water loss responses on D3 (means and SEM). Data are given as delta values.

STUDY II

In study II it was tested if the threshold value for MDBGN in a leave-on product was influenced by the lipid concentration in the vehicle, and if 50 or 100 ppm of MDBGN is a safe level of use in leave-on products.

In treatment of skin diseases such as CD, moisturisers containing preservatives are often used for treatment. The threshold for a reaction to a preservative depends on the penetration of the allergen and also the vehicle. A previous study has shown an increased penetration of Ni when applied after a moisturiser with a high lipid content (70%) (71). Differences in regional penetration of allergens in different vehicles (low lipid and high lipid content moisturisers) have been investigated by Wester & Maibach (17). In study II, the neck was chosen as the test site because of good penetration in this location (17), and since this is a cosmetic-relevant test area for a moisturiser (Fig. 5).

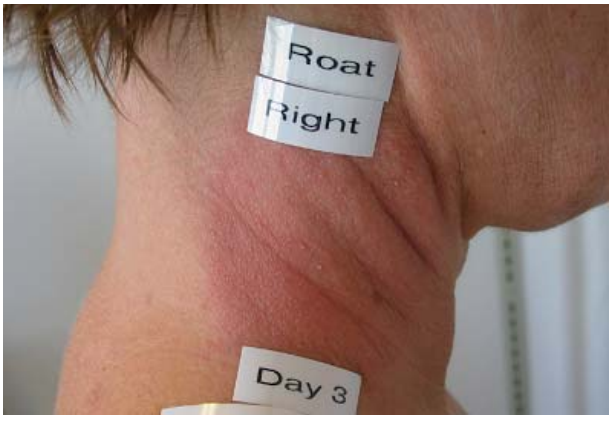


Fig. 5. Vigorous allergic reaction on D3 to the low lipid moisturiser.

Study II showed that the skin response to MDBGN in a low lipid vehicle was greater than MDBGN in a high lipid vehicle. However, MDBGN in the low lipid moisturiser was easier dosed and a greater amount was used, which may be the reason why CD was elicited at a lower threshold than the high fat vehicle. In treatment of CD, moisturisers with added preservatives are used as treatment for this specific skin disease. The threshold for elicitation of ACD is thus lowered when a low lipid moisturiser is applied. This is explained by an easier and increased dosage of the product. Despite participants in study II being carefully instructed to use identical amounts of the two moisturisers, almost all used higher amounts of the low lipid moisturiser. A future study ensuring application of an identical amount of MDBGN in different vehicles is necessary to finally answer the question of whether the threshold value for MDBGN in a leave-on product is influenced by lipid concentration in the vehicle.

The results of study II clearly illustrate that 50 ppm is not a safe level of use for MDBGN in a leave-on product, and that the ban of MDBGN in leave-on cosmetics by the EU-Commission is justified.

STUDIES III AND IV

To date, combination effects of two or several irritants have been only sparsely investigated (69, 71). Use of alcohol-based hand disinfectants and detergent-based soaps is frequent in the health sector. In some studies the use of alcohol-based disinfectants is reported as less irritating than the standard hygienic hand-washing procedure during routine work in clinical wards and intensive care units (92–96). However, other studies have proved disinfectants to be one of the main risk factors for development of HE (97, 98). In studies III and IV it was tested if repeated exposure to a single local irritating agent influences skin barrier function and inflammatory response differently from combined exposure of two skin irritating substances used alternately. It was also tested if skin disinfection with detergent causes more skin barrier disruption and inflammation than skin disinfection with alcohol-based disinfectant (Fig. 6).

The results clearly show that combination of disinfectant/detergent caused less skin irritation than detergent applied alone, probably due to a diminished total amount of detergent exposure. The combined exposure caused an irritant effect, which was increased compared with alcohol disinfectant alone (Fig. 7).

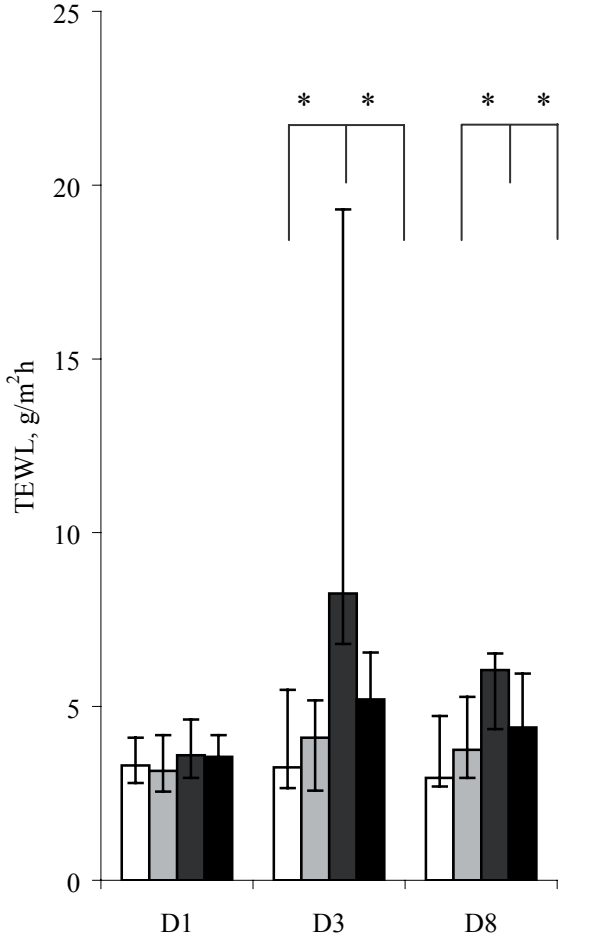


Fig. 6. Comparison of evaporimetric responses between control site, disinfectant, detergent and disinfectant/detergent on D1, D3 and D8 (median values and 25/75 percentiles), *p<0.05. □ Control site, ▒ Disinfectant, ▒ Detergent, ■ Disinfectant + detergent

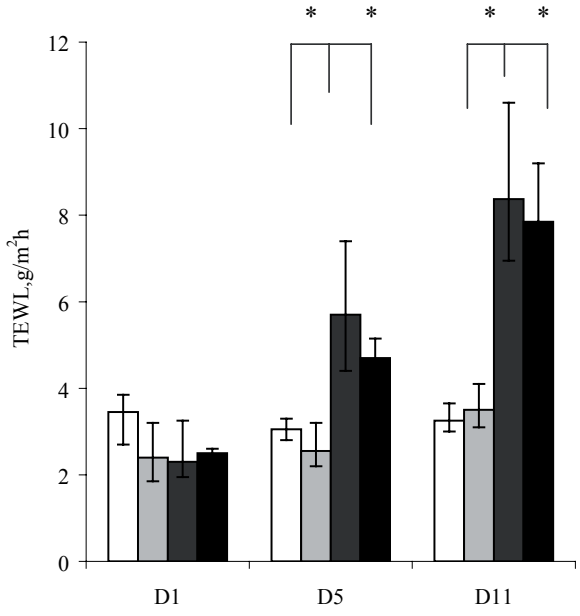


Fig. 7. Comparison of evaporimetric responses between control site, disinfectant, detergent and disinfectant/detergent on D1, D5 and D11 (median values and 25/75 percentiles). * indicates p<0.05. □ Control site, ▒ Disinfectant, ▒ Detergent, ■ Disinfectant + detergent

Clarification of a possible additive or synergistic response could not be concluded in the present design, but could be established in a future study where the same total dose of each irritant is applied in the combined area. However, the aim here was to mimic a realistic exposure assessment. Winnefield et al. (96) also found that alcohol-based hand disinfectants were less irritating than soaps when evaluated by clinical assessment and TEWL, but had no Minoltameter measurements included in the design. Based on results from studies III and IV, alcohol-based skin disinfection is preferable to soap when hands are not visibly contaminated.

In studies III and IV it was also tested if skin exposure to SLS or alcohol-based disinfectant caused hardening after 4 weeks. In study III, where the skin had been exposed to the irritant for only two days, no hardening effect was found. In study IV, where the skin had been exposed to irritants for 10 days, decreased skin reactivity was found at the alcohol solution test site 4 weeks after exposure, when evaluated by colourimetry. Evaluations using TEWL measurements indicated a similar trend for hyporeactivity; however, it was not statistically significant. No indication of hardening was found for skin previously exposed to SLS. The reason that hardening did not develop, as had been expected from earlier studies (99), could be that 4 weeks is too short a period, or that the clinical irritant reaction was too weak. The duration of exposure and the time interval between skin irritation and evaluation of hardening should be extended in future studies.

CONCLUSIONS

The following conclusions may be drawn on the basis of the results presented in this thesis:

COMBINATION OF ALLERGEN AND IRRITANT

Combination of MDBGN and SLS augment the allergic response additively in MDBGN allergic individuals compared to separate exposure to MDBGN.

COMBINATION OF ALLERGEN AND VEHICLE (LOW VS. HIGH LIPID CONTENT)

Skin response to MDBGN combined with a low fat vehicle is higher than that with a high fat vehicle. This is due to a larger amount of low lipid vehicle being used, due to easier application. Further studies are needed using a gram to gram comparison of MDBGN in both vehicles to clarify if the lipid content influences the risk of elicitation of ACD from a leave-on cosmetic product.

CONCLUSIONS CONCERNING MDBGN

50 ppm is not a safe level of use for MDBGN in a leave-on cosmetic product.

CONCLUSIONS ABOUT COMBINATION OF TWO IRRITANTS

Alcohol-based disinfection causes less skin irritation and less skin barrier defect compared to detergent. Use of alcohol-based disinfectant and detergent alternately caused less skin irritation than detergent applied alone.

CONCLUSIONS CONCERNING IRRITANTS/HARDENING

No definitive conclusion can be made about skin reactivity or hardening effect. However, 4 weeks after skin irritation from alcohol solution, decreased skin reactivity was shown. More studies are needed to further clarify hardening.

FUTURE STUDIES

Future research in the field of combined effects should focus on the following areas:

- The combined effect of more allergens (e.g. MDBGN and a perfume ingredient) and more irritants (a skin barrier disrupting irritant and a non-skin barrier disrupting irritant) should be investigated in a clinical experimental study using ROAT. The substances should be applied separately and in combination to ensure that skin sites exposed to one or more substances, respectively, are exposed to the same total amount of each substance. This study will clarify if the combined exposure to irritants and allergens influences skin response, and if it is an additive or synergistic effect.
- The penetration of MDBGN in a vehicle with a low lipid concentration and one with a high lipid concentration should be investigated using a standardised amount of moisturiser (gram to gram comparison) in a randomised clinical experimental study using ROAT. This will clarify how the vehicle influences the risk for elicitation of MDBGN allergy in sensitised individuals.
- Hardening of the skin barrier needs to be investigated further in clinical experimental or intervention studies using different irritants; different degrees of irritation and different time intervals between skin irritation; and evaluation of a hardening effect.
- The impact of hand disinfection with detergents and alcohol solutions should be investigated in a clinical intervention study using realistic exposure test site.

SUMMARY

COMBINED EFFECTS OF ALLERGENS AND IRRITANTS

Contact dermatitis (CD) is a common disease in the population and the most frequently recognised occupational disease in Denmark. CD can be due to either allergy or irritation and many clinical cases are due to a combination of both allergic and irritant contact dermatitis. Some recent studies have indicated an increased skin reactivity from combined exposures to allergens and irritants, and in addition a decreased threshold value for reaction to allergens. Typical examples of combined exposures are consumer products, cosmetics or cleansing products, where numerous contact allergens may be present in combination with detergents. Simultaneous exposure to a combination of two irritants often occurs in "wet work" and an interaction between two different irritants has been reported. Moisturisers containing preservatives are often used for treatment of CD. Many of these preservatives are contact sensitisers and the threshold value for a reaction to the preservative in already-sensitised individuals depends among other things on the potency and penetration of the allergen. Recent studies have indicated that pre-treatment of the skin with moisturisers with a high lipid concentration may facilitate penetration and increase skin response to allergens and irritants.

The present Ph.D.- thesis entails four clinical experimental studies. Participants in the studies were eczema patients previously tested positive to methyldibromo glutaronitrile (MDBGN) and healthy volunteers. For both allergic and irritant reactions patch testing and repeated open application test (ROAT) were used. Visual reading and non-invasive bioengineering techniques (transepidermal water loss (TEWL) and skin colour measurements) were used to evaluate skin reactions.

The irritant sodium lauryl sulphate (SLS), a skin-barrier-disrupting detergent was used as a model irritant together with ethanol (Eth), an inflammatory-generating disinfectant. The model allergen MDBGN, a preservative frequently used in liquid soaps and moisturiser, was used as an experimental allergen.

THE AIMS OF THE THESIS WERE:

1. To evaluate the combined effect of the preservative MDBGN and SLS on the elicitation response of allergic contact dermatitis in MDBGN allergic individuals.

2a. To evaluate if the lipid content of a moisturiser influences the risk of elicitation of MDBGN allergy in already sensitised individuals.

2b. To evaluate if 50 or 100 ppm MDBGN in a moisturiser (leave-on product) can be tolerated by MDBGN sensitised individuals.

3a. To test if alternate application of disinfectant/detergent causes more skin irritation than disinfection and detergent applied alone.

3b. To test if repeated disinfection with detergent causes more skin irritation than repeated skin disinfection with alcohol-based disinfectant.

3c. To evaluate if changes in skin reactivity occur 4 weeks after experimental skin irritation.

The combined exposure to MDBGN and SLS significantly influenced the skin response to MDBGN, which was augmented by a factor 6.4. Combined exposure to MDBGN /low fat vehicle and MDBGN/high fat vehicle showed an increased response to the allergen combined with a low fat vehicle. However, the low lipid vehicle was easier dosed and therefore an increased amount of low lipid moisturiser was used. In addition, a safe level of use for MDBGN in a leave-on product was shown to be less than 50 ppm.

The combined effects of two irritants showed that alternate exposure to alcohol-based disinfectant/detergent showed no increased skin response compared to application of detergent separately. Moreover it was shown that hand disinfection with alcohol-based solution is less skin irritating than hand disinfection with detergent. Indications of decreased skin reactivity 4 weeks after skin irritation with alcohol solution was found, but further studies are needed to elucidate details.

In conclusion, combination of MDBGN and SLS increased the allergic response compared to separate application in MDBGN allergic patients. This finding is important concerning safe threshold values for allergens, since these may be markedly changed in the presence of detergents. Furthermore, it was found that 50 ppm is not a safe level of MDBGN in a moisturiser. This has been part of the documentation supporting the ban of MDBGN in all types of cosmetic products coined by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers.

No increased response was found by alternating the two irritants (alcohol-based disinfectant and detergent) as compared to application of detergent separately. Furthermore, it was confirmed that alcohol-based disinfectant causes less skin irritation than detergent. It can be concluded that in conventional hand washing, the detergent (soap) can be replaced by an alcohol-based disinfectant when hands are not visibly contaminated, and that alternate use of detergent/alcohol solution should not be recommended against. These findings are important with respect to wet work, and may help prevent development of chronic irritant hand eczema.

DANSK RESUMÉ (SUMMARY IN DANISH)

KOMBINATIONSEFFEKTER AF ALLERGENER OG IRRITANTER

Kontakteksem er en almindelig sygdom i befolkningen og den hyppigst anerkendte arbejdsbetingede sygdom i Danmark. Kontakteksem kan være betinget af såvel allergi som af irritation, og mange kliniske tilfælde er kombinerede allergisk og irritativt kontakteksem. Enkelte nyere undersøgelser har indikeret en øget hudreaktion ved kombineret udsættelse for allergener og irritanter, og en nedsat tærskelværdi for reaktion på allergener. Typiske eksempler på kombinerede udsættelser er kosmetiske produkter og rengøringsmidler, hvor flere kontaktallergener og irritanter kan forekomme i kombination. Fugtighedscremer tilsat konserveringsmiddel anvendes ofte i behandlingen af kontakteksem. Mange af disse konserveringsmidler er allergifremkaldende, og tærskelværdien for en reaktion over for konserveringsmidlet hos allerede sensibiliserede personer afhænger blandt andet af potensen og penetrationen af allergenet. Nyere studier har vist, at forbehandling af huden med fugtighedscremer med et højt lipidindhold kan facilitere penetrationen af allergenet og dermed øge hudresponset overfor allergener og irritanter.

Ph.d.-afhandlingen består af 4 klinisk eksperimentelle studier.

Testpersonerne var patienter med kontaktallergi overfor methyldibromo glutaronitril (MDBGN) rekrutteret fra Dermatologisk afdeling K, Gentofte Hospital, samt raske testpersoner. Epikutantest og anvendelsestest, (repeated open application test, ROAT) blev anvendt til at provokere både allergiske og irritative reaktioner. Visuel aflæsning og noninvasive målemetoder som Evaporimetri og Colourimetri blev anvendt til at evaluere henholdsvis det transepidermale vandtab (TEWL) og det inflammatoriske respons (rødme, a* value) på de fremkomne eksemreaktioner.

Som model-irritanter har været anvendt detergenten sodium lauryl sulfat (SLS) (sæbestof) med barriereskadende effekt, ethanol (alkohol) med inflammationsskabende effekt og som model allergen har været anvendt MDBGN, et konserveringsmiddel som hyppigt forekommer i flydende sæber og cremer.

Formålene med afhandlingen var at afklare om:

- Tærskelværdien for en reaktion på konserveringsmidlet MDBGN sænkes og hudens reaktivitet overfor stoffet øges ved samtidig applikation af SLS.
- Om elicitering af allergisk kontakteksem ændres afhængigt af om MDBGN er tilsat en fugtighedscreme/et vehikel med højt lipidindhold sammenlignet med en creme med lavt lipidindhold. Desuden at afklare om 50 eller 100 ppm MDBGN i en fugtighedscreme kan tolereres af MDBGN sensibiliserede individer.
- At undersøge om alternerende brug af alkoholbaseret hånddesinfektion/detergent forårsager mere hudirritation end desinfektion med detergent alene.
- At undersøge om desinfektion med detergent forårsager mere hudirritation end desinfektion med alkoholbaseret opløsning.
- At undersøge om der udvikles en ændret reaktivitet 4 uger efter eksperimentel hudirritation.

Resultaterne viste, at kombinationen af MDBGN og SLS øgede det allergiske respons med en faktor 6.4 sammenlignet med MDBGN alene. Kombineret eksposition for allergenet MDBGN/vehikel (lavt lipid- versus højt lipidindhold) viste et øget allergisk respons med allergen kombineret med vehikel med lavt lipidindhold. Dette var forårsaget af lettere og dermed større dosering af creme med lavt lipidindhold. 50 ppm er ikke et sikkert brugsniveau for MDBGN i creme.

Kombinationen af to irritanter (alkoholbaseret hånddesinfektant/detergent) alternerende gav ikke mere hudirritation end applikation af detergent separat. Huddesinfektion med alkoholbaseret opløsning er mindre hudirriterende end desinfektion med detergent. Indikationer for en mindsket hudreaktivitet 4 uger efter hudirritation med alkoholopløsning blev observeret, men der er brug for yderligere studier til at belyse denne effekt.

Udfra ovenstående resultater kan det konkluderes, at kombinationen af MDBGN og SLS øgede det allergiske respons sammenlignet med separat applikation hos MDBGN-allergiske patienter. Dette fund er vigtigt for reguleringen af tærskelværdier for allergener, da disse tærskelværdier tydeligvis bliver ændret ved kombination med en irritant. Desuden fandtes, at 50 ppm ikke er et sikkert anvendelsesniveau for MDBGN i en fugtighedscreme. Dette fund har været en del af den dokumentation, der forbyder anvendelsen af MDBGN i kosmetiske produkter, udarbejdet af den videnskabelige komité i EU-Kommissionen, the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers.

Der er intet øget respons ved at alternere mellem alkoholbaseret desinfektant og detergent sammenlignet med applikation af detergent alene. Derudover giver alkoholbaseret hånddesinfektion mindre hudirritation og mindre barrieredefekt end detergent. Heraf konkluderes, at konventionel håndvask med detergent kan erstattes med alkoholbaseret hånddesinfektion, når hænderne ikke er synligt kontamineret, og alternerende brug af detergent/alkoholbaseret hånddesinfektion kan med fordel anbefales. Disse fund er vigtige i forbindelse med rådgivning i våde erhverv, og kan være med til at forebygge udviklingen af kronisk irritativt håndeksem.

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