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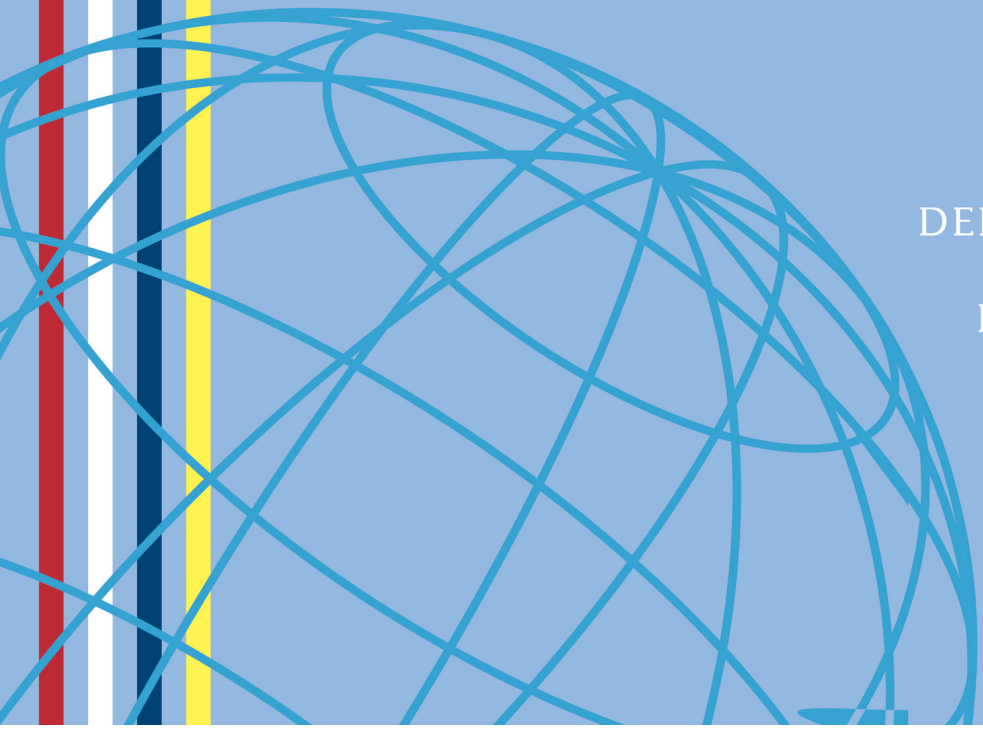

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Development of an in-vivo test method for the evaluation of the effect of anti-irritants in the treatment of irritant contact dermatitis

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Preface

The present thesis entitled *"Development of an in-vivo test method for the evaluation of the effect of anti-irritants in the treatment of irritant contact dermatitis"* was prepared in fulfillment of the criteria for obtaining the Ph.D. degree at the Faculty of Health Sciences, University of Southern Denmark. The project was cooperation between the Department of Dermatology at Odense University Hospital (Klaus Ejner Andersen and Carsten Bindslev-Jensen) and LEO Pharma (Ann Fullerton, and Kathryn Hedegaard).

My very special thanks to Albert and Lori Kligman and all the great people of S.K.I.N. Inc., for three great years in bioengineering Paradise.

The studies presented herein would not have been possible without the help and contributions of the following people: My supervisors Klaus Ejner Andersen, Ann Fullerton, Carsten Bindslev-Jensen, Thomas Kongstad Petersen and for a brief period Mads Bjelke Petersen from LEO Pharma who were always ready with guidance and support and for providing laboratory facilities at the University of Southern Denmark and LEO Pharma A/S.

I am greatly indebted to Kathryn Hedegaard for solving the complex statistics of the studies and for her never ending enthusiasm.

I am truly grateful for technical assistance provided by Pavla Setina (now retired) and Mette Sonne both LEO Pharma A/S and by Ulla Bindslev-Jensen, Kirsten Hammond Andersen, Jette Krapalis and Lisbeth Jensen all from Odense University Hospital.

Last but not least I would like to thank the volunteers who made the studies possible for their interest and participation.

Without financial aid from LEO Pharma A/S and the University of Southern Denmark the studies would not have been possible. My very special thanks to Aksel Jørgensen (now retired) and Jens Hansen both from LEO Pharma A/S for always being there with a helping hand when the economy was getting critical.

Odense, May 2005 *Flemming Andersen*

This thesis is dedicated to my mentor and friend Albert Montgomery Kligman, father of investigative dermatology.
The experimental work is presented in the following seven papers:

Paper I: Andersen F, Hedegaard K, Fullerton A. Differences in response to topical irritants in haired and hairless guinea pigs. Journal of Toxicology – Cutaneous and Ocular Toxicology 2004:23(3):159–171.

Paper II: Andersen F, Hedegaard K, Fullerton A, Bindslev-Jensen C, Andersen KE. Comparison of the response to topical irritants in hairless guinea pigs and human volunteers. Journal of Toxicology – Cutaneous and Ocular Toxicology 2005; 24(1): 31–43.

Paper III: Andersen F, Hedegaard K, Petersen TK, Bindslev-Jensen C, Fullerton A, Andersen KE. The hairless guinea pig as a model for treatment of acute irritation. Skin Research and Technology 2006: Accepted for publication.

Paper IV: Andersen F, Hedegaard K, Petersen TK, Bindslev-Jensen C, Fullerton A, Andersen KE. The hairless guinea pig as a model for treatment of cumulative irritation. Skin Research and Technology 2006: Accepted for publication.

Paper V: Andersen F, Hedegaard K, Petersen TK, Bindslev-Jensen C, Fullerton A, Andersen KE. Anti-irritants I – Effect on acute irritation. Contact Dermatitis 2006. Accepted for publication.

Paper VI: Andersen F, Hedegaard K, Petersen TK, Bindslev-Jensen C, Fullerton A, Andersen KE. Anti-irritants II – effect on cumulative irritation. Contact Dermatitis 2006. Accepted for publication.

Paper VII: Andersen F, Hedegaard K, Petersen TK, Bindslev-Jensen C, Fullerton A, Andersen KE. Comparison of the effect of glycerol and triamcinolone acetonide on cumulative skin irritation in a randomized trial using human volunteers. Submitted for publication.

The intention of this thesis is to present a theoretical background for the studies followed by a presentation and discussion of the experimental work. The methodology of the experimental work is primarily described in the papers.

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LIST OF ABBREVIATIONS

AI	Anti-irritant
AUC	Area under the curve
CGP	Clipped and depilated guinea pig
HLGP	Hairless guinea pig
HV	Human volunteer
TEWL	Transepidermal water loss

1. INTRODUCTION

Contact dermatitis defined as eczematous skin reactions due to contact with allergens or irritants, is a common dermatological problem. Contact dermatitis may affect all skin areas and is often located to the hands, causing severe nuisance for the sufferer. Patients are often exposed to both irritants and allergens, resulting in contact dermatitis of mixed type. Irritant contact dermatitis (ICD) appears to be more frequent than allergic contact dermatitis. Common causes to ICD are wet work, dirt and cleaning agents (1;2). As ICD may become chronic and disabling prevention by mandatory exposure control, legislation, education and personal protection is *de rigeur*.

Topical corticosteroids is an important remedy in the treatment of contact dermatitis both in the acute and chronic state. Impairment of the stratum corneum barrier is a common feature of contact dermatitis allowing penetration of external substances that may contribute to increased irritation. As topical corticosteroids have been shown to disrupt the stratum corneum barrier (3) they may not be the best treatment modality in some instances of ICD.

Goldemberg defined a diverse group of chemical entities called anti-irritants as "agents that used in conjunction with skin or eye irritants, reduces their irritant potential sufficiently so that they can be tolerated when applied to the body" (4). Increasingly it is claimed that anti-irritants may "sooth" or "heal" sensitive or irritated skin. However, the proof for these claims is often circumstantial.

New anti-irritant chemicals, with an effect in ICD should be developed and tested. However, clinical studies of new products for contact dermatitis will often require large sample sizes due to the complexity of the disease. In the early phase of product development, relevant human experimental models predictive of contact dermatitis will therefore be important tools in order to obtain a fast proof of concept.

2. THEORY AND BACKGROUND

2.1 CUTANEOUS IRRITATION

The risk of experiencing cutaneous irritation is a part of every day life. From we get up in the morning till we go to bed at night we are exposed to potentially irritant chemicals. Chemical irritants are a very heterogeneous group of substances exhibiting different irritant potentials, morphology and biochemistry (5–9). Skin reactivity however not only depends on irritant chemistry but also on exogenous factors, such as mode of exposure and regional differences, as well as endogenous factors such as race and sex (8).

For an irritant to be used in dermatological experiments the following criteria should ideally be fulfilled (5): The irritant shall elicit reaction in about 90% of the population, give reproducible results, should not exhibit systemic toxicity or be a carcinogen. The irritant should not be volatile and the pH should not be extreme. Furthermore the irritants should be chemically well-defined (i.e. not a mixture) and it should not cause cosmetic inconveniences to volunteers such as scarring and staining.

Experimental irritants have been divided into two major groups: corrosive and non-corrosive irritants. Corrosive irritants elicit impairment of the water barrier function, with increased transepidermal water loss, even in weak reactions characterized by faint redness, whereas non-corrosive irritants do not (10).

2.1.1 Model irritants

Two model irritants sodium lauryl sulfate(SLS) and nonanoic acid (NON) fit most of the above criteria and are used as standard irritants. As they furthermore complement each other as SLS is corrosive and NON is non-corrosive, they were chosen as model irritants for the studies described herein (11;12).

2.1.2 Sodium lauryl sulfate

Sodium lauryl sulfate (SLS) is an anionic detergent frequently used in cosmetics, toiletries and topical dermatological formulations. SLS is commonly used as a model irritant, the effect on the skin depends of several factors including concentration, type of application, species etc. Stratum corneum lipids are not significantly altered by SLS. However lipid synthesis appears to be impaired, this is attributed to SLS affecting the basal cells of the epidermis. Histologically the epidermis shows parakeratosis, spongiosis and formation of intracellular vacuoles and lipid accumulation, in strong reactions necrosis can be observed. Keratinocytes are stimulated causing upregulation of factors such as ICAM-1 and IL-1. In the dermis an inflammatory infiltrate is observed consisting mainly of $CD4^+$ cells and neutrophils (11;13).

Clinically the reaction is characterized by erythema. In case of strong reaction infiltration, erosions and even vesicles and pustules can also be seen. Scaling and fissuring is seen following repeated applications (cumulative irritation) and during the late phase of the acute reaction (11).

2.1.3 Nonanoic acid

Nonanoic acid is a long-chain fatty acid, which is insoluble in water but readily dissolves in alcohols. It is used in a variety of industries including production of plastics and pharmaceuticals. For irritant testing NON is typically dissolved in n-propanol or isopropanol (12).

The mechanism of action is unknown. NON may directly affect components of the stratum corneum through a detergent-like action disturbing membranes and disrupting the lipid phase (12).

Histologically the reaction is characterized by infiltration of Langerhans cells ($CD1^+$ cells). Clinically the reaction is characterized by a dose-dependent erythema and edema, followed by formation of a papery film of plasticized stratum corneum. In the late phase transient brown hyperpigmentation can be seen (12).

2.2 THE ANTI-IRRITANT CONCEPT

Cosmetic chemists working with formulating skin care products in the early 1960'ies realized that the irritant potential of known eye and skin irritant components of formulations varied greatly depending on the other components of the formulation. Apparently some excipients actively reduced the irritant potential of other excipients. Goldemberg described these agents as "anti-irritants" (AI), defined as "agents that used in conjunction with skin or eye irritants, reduces their irritant potential sufficiently so that they can be tolerated when applied to the body" (4).

Three possible mechanisms of actions were postulated (4;14).

I) Chemical complex formation, exemplified by the elimination irritancy of menthol with amphoteric imidazoline surfactants.
II) Prevention of complete contact with skin, by using thickening agents such as methyl cellulose to reduce spread of the irritant, or by applying strontium salts preventing penetration of the irritant.
III) Blocking of skin-reactive sites, for instance by applying oily substances to the skin prior to application of aqueous irritants.

Since then an increasing number of agents, demonstrating inhibition of for instance of the arachidonic acid cascade, such as (–)-α-bisabolol have been included in the anti-irritant community (15). Despite Goldemberg insisting, that anti-inflammatory agents were not to be included in the AI categories, perhaps a fourth category should be devised:

IV) Anti-inflammatory effect, exemplified by (–)-α-bisabolol.

Goldemberg did not intend that anti-irritants should be used as treatment modalities for preexisting skin irritation. However ingredients in cosmetic formulations are increasingly being touted as anti-irritants intended to reduce existing skin irritation (16). The vast majority of new alleged anti-irritants are derived from plants, for instance extracts from Calendula, Arnica, Hibiscus, Gingko, oat, raspberry or Schizandra. Proof is mainly from in-vitro studies and occasionally from acute irritation models aimed at demonstrating protective properties (17–19). For instance, glycyrrhetinic acid from licorice root is known to inhibit skin 11beta-hydroxysteroid dehydrogenase, an enzyme that deactivates corticosteroids, further glycyrrhetinic acid appears to exhibit anti-inflammatory properties (20–24).

2.2.1 Selection of anti-irritants for the Ph.D.-study

Prior to study start a Medline search was performed for chemicals with alleged anti-irritant properties fulfilling the following criteria. The potential anti-irritants should be well-defined and purified chemicals. The chemicals should be safe to use in humans and hypo-allergenic. Ideally the substances should have shown clear treatment effect in human models of irritation, if not then effect in animal models, substances that had only exhibited effect in in-vitro models were to be the last choice. The following chemical-groups were chosen for discussion:

- Ion channel modulators (exemplified by nifedipine and ethacrynic acid)
- Barrier stabilizing agents/lipid donors (exemplified by canola oil)
- Anti-inflammatory agents (exemplified by (–)-aðbisabolol)

From each group one agent was chosen, as topical safety of nifedipine was well-documented this substance was chosen from the group of ion channel modulators.

As the humectant glycerol had been the only substance with positive effect in the first 4 studies (Papers I–IV) we decided to include it in the group of model anti-irritants.

2.2.2 Nifedipine

Nifedipine belongs to a group of pharmaceuticals called "calcium channel blockers" (CCB), primarily used as antihypertensive agents, working by inhibiting the flux of calcium ions into cells (25).

In vitro studies have shown, that cells participating in the inflammatory response depend on calcium as a transmembranal messenger, and that CCBs inhibit both the afferent and efferent inflammatory response (26–34).

It has been shown that UV-radiation destroys the calcium channel blocking effect of nifedipine (35). Chang et al demonstrated that nifedipine despite UV-radiation and loss of calcium channel blocking effect was still able to inhibit PLA2-activity (36). Thus it appears that the mechanism of action on the inflammatory response is, at least partially, unrelated to the CCBs effect on calcum channels (37;38).

Several animal and human studies have demonstrated inhibition of cutaneous contact sensitivity by CCBs, including nifedipine, administered either perorally, parenterally or topically (39–45). Anti-irritant effect of calcium channel blockers has been demonstrated in various animal models (46–48). Concomitant application of nifedipine had no effect on irritation caused by Captopril gel in a rabbit model (49).

Regarding safety: nifedipine, applied topically, has been studied as a treatment modality for anal fissures without noticeable adverse effects (50;51). Furthermore attempts at developing a transdermal delivery system for nifedipine has failed due to too low drug flux (52). Thus nifedipine was deemed safe to use as a topical anti-irritant in human volunteers.

Source: Ph.Eur., Siegfried CMS AG, Kirchberg, Schweiz

2.2.3 (–)-α -bisabolol

(–)-α -bisabolol a cyclic monounsaturated sesquiterpenic alcohol is found in the essential oil of *Matricaria recutita*. Concentrations in skin care products are in the range of 0.1–1.0%, most commonly between 0.2–0.5%.

(–)-α -bisabolol is used extensively in skin care products as an anti-phlogistic or anti-irritant agent. Anti-irritant effect has been demonstrated in animal and human studies, effect is ascribed to blocking of the enzymes 5-lipoxygenase and cyclooxygenase of the arachidonic acid cascade (53–55).

Source: min. 95% pure, Cosnaderm, Ladenburg, Germany

2.2.4 Canola oil

Canola oil is a rape seed oil from rape varieties developed in Canada by selective breeding to obtain a variety with a low content of saturated fatty acids (especially erucic acid). Canola oil contains 55% oleic acid, 25% linoleic acid, 10% alpha-linolenate and 4% saturated fatty acids (56;57).

Lodén has demonstrated anti-irritant effect of canola oil against sodium lauryl sulfate induced acute irritation. Lodén speculated that the effect might be due to canola oil constituents acting as lipid donors and anti-inflammatory agents (58;59).

Source: Lipex Canola-U, Karlshamns AB, Karlshamn, Sweden

2.2.5 Glycerol

Glycerol is a trihydroxy alcohol obtained by saponification of fats and oils, used as an emollient and humectant in topical formulations, a wide range of effects of glycerol on the stratum corneum has been documented:

In a stratum corneum model glycerol prevents the transition of stratum corneum lipids from the liquid crystalline phase to the solid crystalline phase (60;61). Glycerol is a humectant exhibiting dose-response (62;63). Glycerol facilitates digestion of desmosomes, thus enhancing removal of scales (64). Long term use of glycerol changes the mechanical properties of the stratum corneum, making it more elastic (65;66). Glycerol reduces cutaneous roughness (62;67). Glycerol improves cutaneous barrier function when applied prior to or after irritant exposure (68–70). Glycerol enhances penetration of hexyl nicotinate (68).

Source: Ph.Eur., Karlshamn Tefac AB, Karlshamn, Sweden

3. OWN STUDIES

3.1. AIM OF THE THESIS

The thesis encompasses two series of studies (Fig. 1). The first was designed to evaluate the hairless guinea pig (HLGP) as a cutaneous model for the evaluation of new topical formulations in regard to tolerability and effect on acute and cumulative irritation (Papers I–IV). In the European Union the use of animal models for the evaluation of finalized cosmetics and skin care products has been banned, and testing of potential new cosmetic ingredients in animal models will be banned from the year 2009 unless alternative methods cannot be developed (71). Although animal models are still allowed in drug development they should be re-evaluated and compared to alternative in-vitro and human methods.

The second aimed at developing an in-vivo model for efficacy testing of anti-irritant substances as treatments of acute and cumulative irritation compared to a topical corticosteroid (Papers V–VII). By using a small sample size and a rather large minimum detectable difference only potent anti-irritants were expected to be found.

3.2 STATISTICAL ANALYSES

The structure of the data is based on repeated measurements. For simplicity the following overview is based on the final three studies. Statistical analyses was performed by statistician Kathryn Hedegaard, LEO Pharma A/S.

In the acute study estimation of dose-related treatment effect and identification of any time-related differences between the treatments was of interest (Paper V). In the forearm wash test focus was on estimation of any overall treatment effects as well as identification of any time-related differences between the treatments (Paper VII).

For the acute irritation study and the forearm wash test a statistical method modelling the whole profile was chosen, i.e. a repeated measures model 'Covariance Pattern model', as this enables flexible specification of the correlation between observations relating to the same treatment within a particular volunteer (72).

A model giving the best fit for the data in question was then chosen. As the main factor of interest in these studies "time-related differences between treatments" was not significant a simpler ANCOVA (Analysis of Covariance) model analyzing the response on the final day was used to examine for treatment effects. Factors of interest and covariates, such as time-related differences between treatments" and "position of treatment site" were modelled as fixed effects.

Several treatments were applied to the same volunteer. The measurements belonging to the same volunteer will be more closely correlated than observations between different volunteers. Therefore, although the individual response levels amongst the different volunteer was not of interest, it was necessary to allow for this in the specified variance structure of the models. This was made by including volunteer as a random effect in the repeated measures model and ANCOVA model, i.e. a mixed model was used (73).

The validity of the assumption of normality which underlies the mixed model was checked by examining normal plots of the standardized residuals. Where necessary, the data was log-transformed or outliers removed.

In the comparison of treatments within the final models for the acute irritation study and the forearm was test, adjustment for inflation of the Type I error rate resulting from multiple comparisons was made using the Tukey-Kramer method.

Due to poor model fits for the total clinical score data, the results from the Mixed Models were compared with the results obtained using the non parametric Wilcoxon.

In the cumulative irritation study identification of any differences between the effects of the various anti-irritants at Day 11 was of primary interest. Biometric measures were assessed at baseline (Day 0), Day 4 and at end of treatment (EOT) on Day 11. The analysis of choice was therefore Analysis of Covariance (ANCOVA), thereby allowing adjustment of the EOT values for the baseline values. Clinical score was assessed daily. The daily clinical score measurements were summarized by constructing the summary measure Area under the Curve

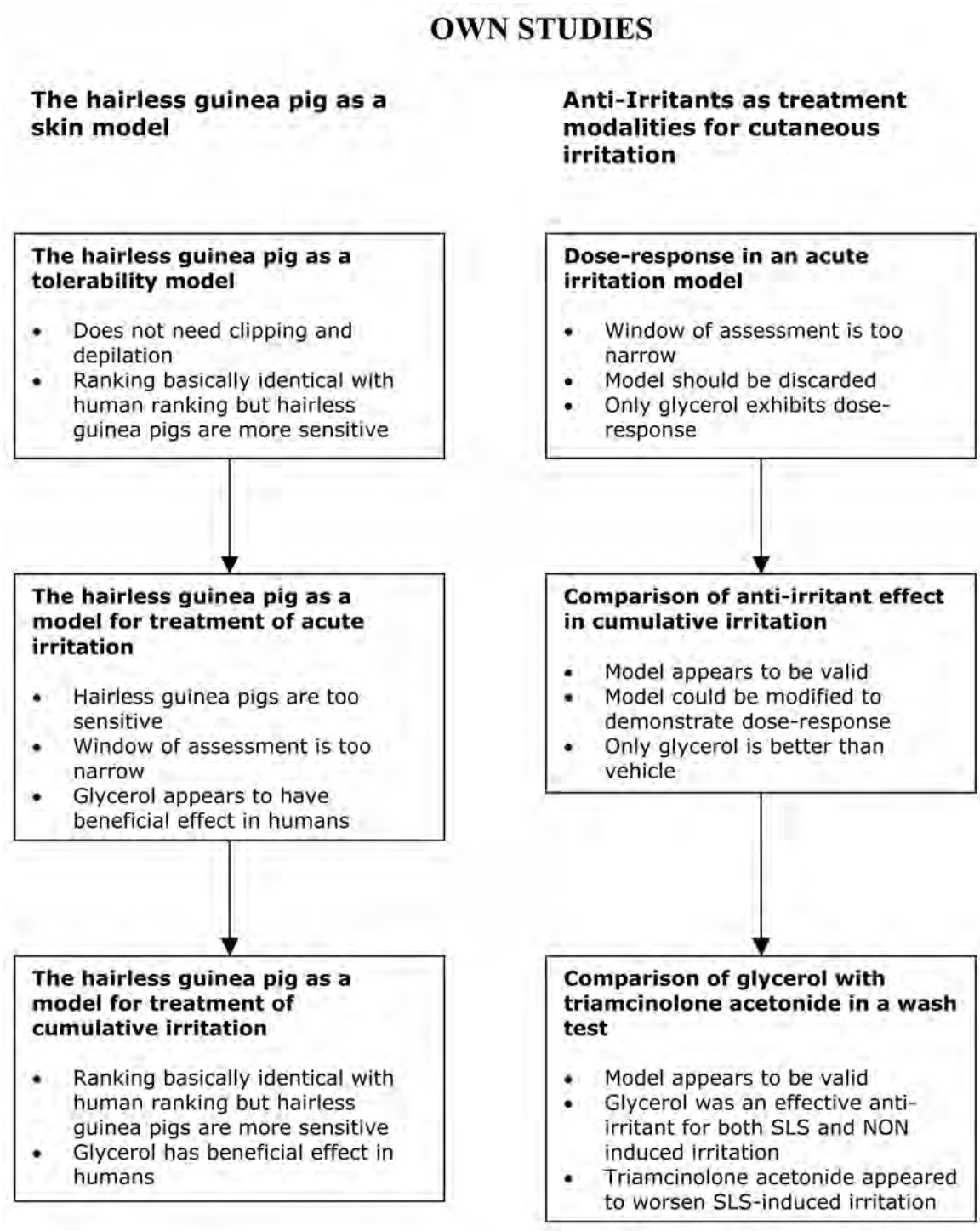

Fig. 1. Flow diagram of own studies

(AUC). This summary measure was then modelled using Analysis of Variance (ANOVA).

The validity of the assumptions of normality and equal variance that underlie the ANCOVA and ANOVA models were checked by examining normal plots of the standardized residuals as well as performing the Shapiro-Wilk and Bartlett Tests. Where necessary, the summary measures were logarithmically transformed or outliers removed.

In both the ANOVA and ANCOVA models, the factors of interest – treatments and position of test site on the arm – were modelled as systematic factors (fixed effects). Significance was assumed at the 5% level (i.e. factors were retained in the model if their p-value < 0.05).

All treatments were applied to the same volunteer. Measurements belonging to the same volunteer will therefore be correlated, whilst measurements from different volunteers will not. Thus, it was necessary to adjust the variance structure of the models to allow for the different degree of correlation among the observations. This was done by including volunteer as a random effect in both the ANOVA and ANCOVA models again a mixed model was used.

In the comparison of treatments, adjustment for inflation of the Type I error rate resulting from multiple comparisons, has been made in the mixed model using the Tukey-Kramer method.

All analyses have been performed using Proc Mixed, SAS version 8.2. The Kenward-Roger method for adjusting the standard errors and degrees of freedom obtained has been used.

3.3 The hairless guinea pig as a cutaneous model

Clinical studies of new treatments for contact dermatitis in humans tend to require large sample sizes due to the complexity of the disease. In the early phase of product development experimental models of irritant contact dermatitis are necessary as screening tools and for proof of concept.

The European Centre for the Validation of Alternative Methods sponsors validation studies on in vitro tests for skin irritation, so far three models have been approved as replacements for the in vivo skin corrosivity test to be used for hazard identification and classification of corrosive potential. These models do so far not appear to be suited for the testing of complex low-grade irritants such as vehicles for pharmaceutical formulations. Furthermore this type of in vitro models would classify for instance a non-irritant neurotoxin as safe, whereas in an animal model the neurotoxin would have killed the animals (74).

In short the need for animal models will exist until in-vitro methods are developed that mimic the responses of a whole organism. There are numerous animal models for skin inflammation and irritation. The end point is typically erythema or edema. The outcome will vary with species, irritant, method of application and the treatment studied and it is not necessarily possible to extrapolate the results to humans (75).

The lack of consistency between animal and human studies necessitates the re-evaluation of existing animal models by comparing them with alternative in-vitro and human methods.

The euthymic hairless guinea pig is widely used in cutaneous studies as it can be used without the potentially damaging effect on the stratum corneum of clipping and depilation. The hairless surface offers better contact with the test products and eliminates the interference of regrowth of hair with visual and instrumental evaluations (76). The sensitivity of the hairless guinea pig to topical irritants and cutaneous histological resemblance to human skin has contributed to its use (77;78).

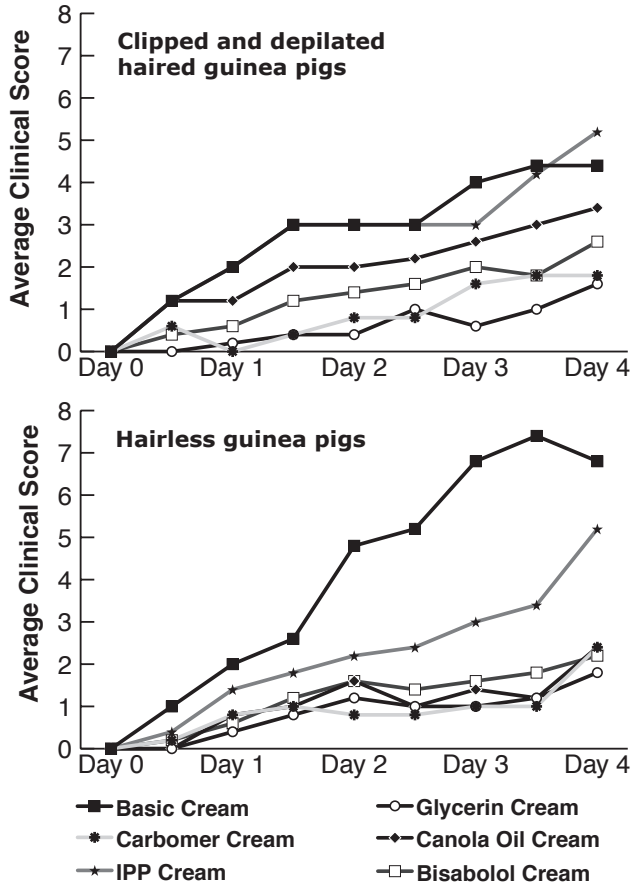

Fig. 2. Development of irritation assessed clinically from Day 0 (baseline) to Day 4

Studies have shown a response to allergens and simple irritants comparable to that of the haired guinea pig but with differences depending on substance and concentration used (79–81). But the effect of complex weak topical irritants, such as skin care formulations, has not previously been studied.

In the ensuing studies 6 model formulations, developed by LEO Pharma A/S, were selected. Based on LEO Pharma internal reports (unpublished data) they were expected to give a graded irritant response in the hairless guinea pig.

As positive vehicle control was chosen "Basic Cream", a known irritant to hairless guinea pigs. As negative vehicle control was chosen the well-tolerated "Carbomer Cream". The "Carbomer Cream" was used as the basis for 4 more formulations: the "IPP Cream" made by adding the penetration enhancer isopropyl palmitate (IPP), known to be irritant to guinea pigs; "Canola oil cream" and "Bisabolol cream" were made by adding Canola oil and (–)-α-bisabolol, both safe and published anti-irritants used in food industry and cosmetics. These two formulations were expected to be tolerated as well as or better than the parent compound "Carbomer Cream". Finally a "Glycerol Cream" was formulated to study the effect of a humectant on tolerability.

3.3.1 The hairless guinea pig as a tolerability model (Papers I and II)

Determining the irritant potential of new topical formulations is an important part of the safety assessment program. The first

Table 1 Tolerability of skin care formulations in guinea pigs

	AUC (clinical score)		TEWL		Colorimetry (a*-parameter)	
Tolerability	best	least	best	least	best	least
CGP	D B F E C A		D F B E A C		D A B E F C	
HLGP	D B F E C A		B D F E A C		B D F E C A	

AUC area under the curve, TEWL transepidermal water loss, CGP clipped Dunkin-Hartley guinea pigs and HLGP hairless guinea pigs. Underlining of two formulations means, that they are not significantly different.
A: Basic Cream, B: Carbomer Cream, C: IPP Cream, D: Glycerol Cream, E: Canola Oil Cream; F: Bisabolol Cream.

An Example: The TEWL results for HLGP (B D F E A C) can be interpreted as follows: After adjustment for the baseline (Day 0) values, the order B D F E A C indicates that the level of response to B was less than that of D, whilst the response to D was less than that to F etc. The underlining of B, D, and F, and that of A and C, however, indicates that with statistical testing, the differences in response level seen between B,D and F were not statistically significant and nor was the difference in response level seen between A and C. Therefore, the statistical ranking in terms of which formulation is most well-tolerated is, as assessed by TEWL in HLGP:
1st-B, D, F; 2nd-E; 3rd-A and C.

study compared the tolerability of the 6 formulations in clipped and depilated haired guinea pigs with hairless guinea pigs.

In Fig.e 2 the development in average cutaneous irritation over time is shown. For both CGP and HLGP, all the formulations induced some clinical changes during the course of treatment. In the hairless guinea pigs the Basic Cream produced a much larger response than the IPP Cream, there was little difference in the response obtained with the remaining formulations. For the haired guinea pigs, no difference was seen between the Basic Cream and the IPP cream, but a better differentiation between the remaining formulations was seen.

The statistical ranking of the 6 formulations revealed that by clinical assessment and measurement of TEWL both strains found Basic Cream (positive control) and IPP Cream to be the least tolerable formulations. Both strains ranked the Canola Oil Cream as the third least tolerated skin care formulation in the series. The remaining 3 creams, including Carbomer Cream (negative control), were in general well-tolerated in both strains inducing only minor irritation.

As the positive and negative controls were ranked as expected beforehand the ranking of the remaining formulations was considered reliable. No differences were found between the 3 formulations in the hairless guinea pig, though in the haired guinea pigs, Glycerol Cream was significantly better tolerated than Bisabolol Cream (Table 1).

The differences in the response pattern obtained with the well-tolerated formulations are, however, expected to be of no importance in humans as the guinea pig in general has a very susceptible skin compared to human skin.

With regard to colorimetry, increases in the a*-parameter as an indication of erythema due to inflammation was found for HLGP, whereas a decrease in the a*-parameter leading to a markedly different ranking of the SCF was found for CGP. This was not due to a reduction of erythema but due to the fur of CGP growing back resulting in the reflection of the colorimeters light from a white fur coat. Colorimetry therefore appears to be unsuitable for the evaluation of cutaneous irritation in the CGP over a period of days as regrowth of fur will obfuscate the underlying erythema. Repeated clippings would have removed this source of error, but repeated clippings would on the other hand induce barrier damage leading to another and potentially worse source of error.

In conclusion both models may be used as tolerability models, however the hairless guinea pig appeared to be more suitable, due to the avoidance of clipping and depilation, which is both time-consuming and may affect the non-invasive measurements of skin color. A factor to take into consideration is that a hairless guinea pig priced at about DKK 1200 is roughly 4–5 times more expensive than a haired guinea pig. Also lately the European stocks of hairless guinea pigs are suffering from viral infections, limiting the availability of these animals.

To ensure the relevance of the data obtained in the guinea pig model we redid the study, this time comparing the hairless guinea pig with human volunteers. Intact human skin is very resilient to weak irritants and even under occlusion, in the commonly used human repeat insult patch test model, it may be necessary to apply the product daily for as long as 2–3 weeks before noticeable irritation occurs (82). To circumvent these pit falls the Human Chamber Scarification Test was chosen as human tolerability model. By scarifying the skin prior to application of test products a fast response is obtained even from minor irritants, by using a positive and negative control in the array of products a reproducible ranking of the test products is obtained (83–85).

Applying the test material on scarified skin leads to the following advantages: the method becomes more sensitive and takes shorter time than traditional use tests or occluded provocation studies (85). The human chamber scarification test is a

reproducible, exaggerated exposure skin irritation test developed to rank the irritancy potential of products using SLS and paraffin oil as standard controls (83;84). The reactions in the chamber scarification test tend to start and develop in the scratches, making the objective scoring by standard bioengineering methods impractical; instead a 4 point clinical ranking scale is used.

The guinea pig is highly sensitive to weak topical irritants and may respond to topically applied ointment bases with thickening of the epidermis (86). Subsequently it is not necessary to apply topical formulations under occlusion much less to scarify the skin prior to application. The docile temperament of the HLGP facilitates the use of non-invasive bioengineering methods for the objective evaluation of skin reactions without the use of anesthesia.

The results obtained in the hairless guinea pig model were consistent with the results obtained in the previous study (Compare Tables 1 and 2).

In the human chamber scarification test, the positive and negative controls gave reactions as expected, and therefore the results for the skin care formulations were considered reliable. All formulations were tolerated equally well, at a level similar to that of the negative control, with the clinical score rising slightly on the first day and remaining stable thereafter. Although the positive vehicle controls, the Basic Cream and the IPP Cream produced the highest AUCs these differences were not statistically significant (Fig. 3 and Table 2).

In conclusion, the hairess guinea pig can be used as a sensitive screening model. Negative results in guinea pigs are predictive of good tolerability in humans; however positive results in the guinea pigs do not necessarily indicate that a topical formulation cannot be used in humans.

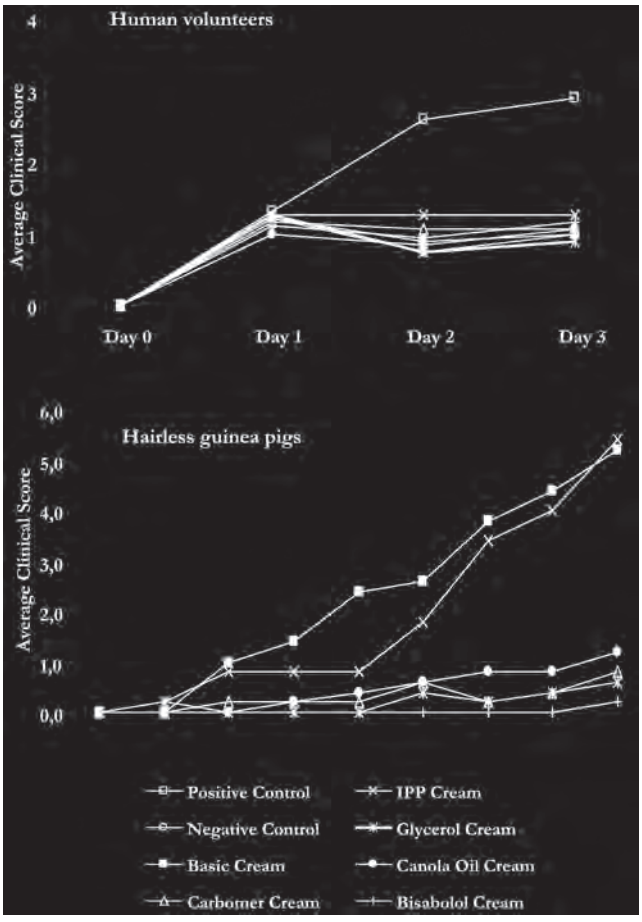

Fig. 3. Development of irritation assessed clinically

Table 2. Tolerability of skin care formulations in hairless guinea pigs and human volunteers

	Clinical Score		TEWL		Colorimetry (a*-parameter)	
	AUC					
Tolerability	best	least	best	least	best	least
HLGP	F D B E C A		D F B E A C		D B F E A C	
	LOG_{10}AUC					
HV	*N* E F D B A C *P*		Not measured		Not measured	

AUC area under the curve, TEWL transepidermal water loss, HLGP hairless guinea pigs and HV human volunteers, *N* and *P* denote the negative and positive controls used in the chamber scarification test. Underlining of two formulations means, that they are not significantly different.
A: Basic Cream, B: Carbomer Cream, C: IPP Cream, D: Glycerol Cream, E: Canola Oil Cream; F: Bisabolol Cream.

An Example: The Clinical Score (AUC) results for HLGP (F D B E C A) can be interpreted as follows: The order F D B E C A indicates that the level of response to F was less than that to D, whilst the response to D was less than that to B etc. The underlining of F, D, B and E, and that of A and C, however, indicates that with statistical testing, the differences in response level seen between F, D, B and E were not statistically significant and nor was the difference in response level seen between C and A. Therefore, the statistical ranking in terms of which formulation is most well-tolerated is, as assessed by AUC in HLGP:
1st-F, D. B and E; 2nd-C and A.

3.3.2 The hairless guinea pig as a model of treatment of cutaneous irritation (Papers III and IV)

Two aspects may be considered when evaluating a skin care formulation. One aspect is the tolerability of the formulation on normal and diseased skin. The other aspect is whether the skin care formulation itself is able to reduce inflammation or irritation induced by other exogenous substances or whether it aggravates the irritation by these by facilitating their penetration.

In the two last studies in this series the effect of the 6 skin care formulations as treatment modalities for acute and cumulative cutaneous reactions was studied in hairless guinea pigs and human volunteers.

Acute irritation

In the third study acute irritation was in induced in guinea pigs and volunteers by 24 h occlusive tests with 1% sodium lauryl sulfate aq. (SLS) in both guinea pigs and volunteers. Further, in the human volunteers acute irritation was also induced with nonanoic acid in n-propanol (NON). The irritant concentrations and sample sizes were chosen based on pilot studies.

The irritant reactions were treated twice daily with the formulations from the time of removal of the patches. As the IPP Cream and the Basic Cream caused severe irritation in the guinea pig tolerability models these formulations were not tested in the hairless guinea pigs.

Guinea pigs were randomized to treatments using a balanced incomplete block design (BIBD): 5 treatments were given, 4 skin care formulations and "no treatment", but only 4 patch test sites were available on each guinea pig. In humans the 7 treatments (6 skin care formulations and "no treatment") were applied to 7 patch test sites on each volar forearm using a latin square design (87).

Reactions were evaluated clinically and by noninvasive methods.

The baseline results in the human model demonstrated the fundamental differences between the two model irritants. The NON reaction was characterized by acute inflammation with minimal barrier affliction whereas the SLS reaction was mainly characterized by barrier damage. This accounts for the high clinical scores with NON, due to erythema and edema, compared to SLS, as well as the high TEWL values following SLS exposure compared to NON exposure.

The outcome of the human acute irritation study mainly differed from the guinea pig study in the way that the glycerol cream was potentially better than No treatment rather than worse.

The inability of the acute model to demonstrate clear proof of the efficacy of known anti-irritant substances, i.e. (–)-aδ-bisabolol and canola oil, could in part be attributed to the small window of opportunity in which to demonstrate efficacy. In the previous pilot study the irritated skin was almost back to baseline within a week after removal of the patches.

Cumulative Irritation

A more relevant model for efficacy-studies of potential anti-irritant treatments than the one-time occlusive patch test might be a cumulative irritation model (88;89).

Three main groups of cumulative irritation models exist: repeated occlusive tests, repeated open tests and wash tests. Wash tests are mainly used as a means of testing detergents for irri-

Table 3. Effect of skin care formulations on acute irritation in hairless guinea pigs and volunteers

	Clinical Score		Colorimetry a*-parameter		TEWL g/m^{2}h		Hydration µS	
Efficacy	best	worst	best	worst	best	worst	best	worst
HLGP SLS	0 G P B C #		0 G P C B		0 G B P C #		Not measured	
HV SLS	P 0 G C F B I		0 P C G F B I		G 0 F B C P I		G 0 F B P C I #	
HV NON	I B C F G P 0		B P F 0 C I G		G 0 P B F C I #		0 C G P B F I	

Underlining of two formukatuion means, that they are not significantly different ($p < 0.05$). An Example:
The TEWL results for HLGP (0 G B P C) can be interpreted as follows: The order 0 G B P C indicates that the effect of No treatment was better than G, whilst the effect of G was better than B etc. The underlining of formulations indicates that with statistical testing, neither the differences in response level seen between 0 and G, nor between B and P were statistically significant. Therefore, the statistical ranking in terms of which formulation is the most effective treatment is, as assessed by TEWL in HLGP: 1st-0 and G; 2nd B and P; 3rd –C.

means that there were time-related differences between the treatments (i.e. the interaction Treatment*Day was significant, and the statistical ranking presented is that found on Day 4.

0 denotes No treatment, F basic cream, P carbomer cream, I IPP cream, G glycerol cream, C canola oil cream, B bisabolol cream, HLGP hairless guinea pigs, HV human volunteers. F and I were not tested in HLGP due to low tolerability.

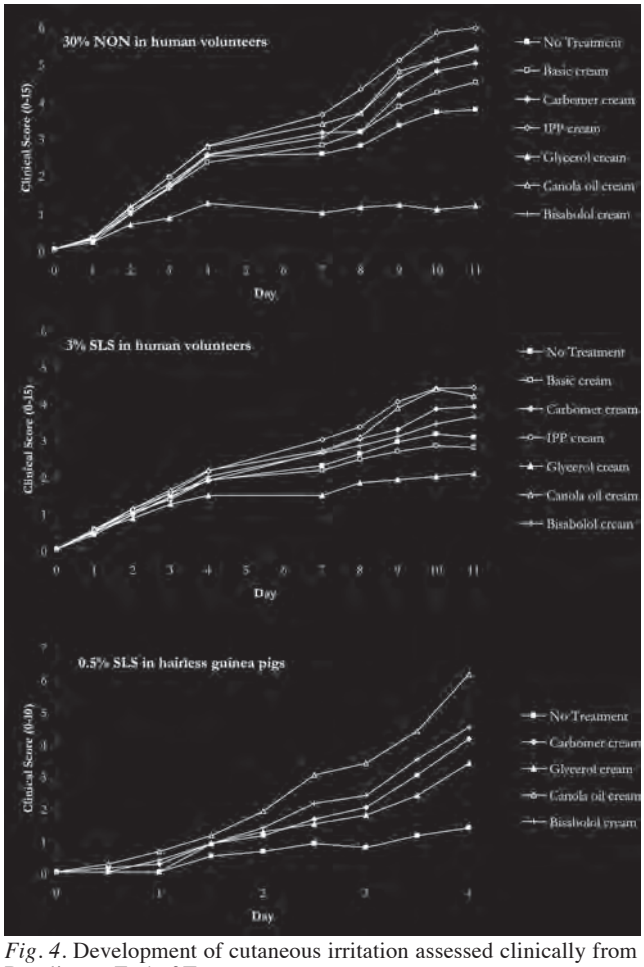

Fig. 4. Development of cutaneous irritation assessed clinically from Baseline to End of Treatment

tancy and would need the development of a new model to fit our needs.

Tupker and co-workers compared the ranking of detergents in one-time occlusive, repeated occlusive and repeated open tests following the guidelines on SLS exposure tests from the European Society of Contact Dermatitis (11;89). They found that the one-time occlusive test and the repeated occlusive test mimic the situation where the skin is occluded after exposure to detergents, i.e. wearing ineffective gloves or applying gloves on detergent soaked skin. The repeated open test mimics the most common situation in which uncovered skin is exposed to detergents several times daily.

In the fourth and final comparative study between humans and guinea pigs we decided to use a repetitive open model as suggested by Tupker.

Inducing cumulative irritation in the guinea pigs using an open model similar to the one used in humans would have required the use of anesthesia on a daily basis with the risk of inducing tolerance to the anesthetic, dehydration etc. Thus we decided to use a semi-occluded model with a minimum effect on the daily life of the guinea pigs.

The choice of irritant concentrations and sample sizes were based on pilot studies. All 6 formulations were tested as treatment modalities in humans, the Basic Cream and the IPP Cream were not tested in the guinea pigs due to the low tolerability.

The results from the cumulative irritation guinea pig model were in concordance with the ranking of the formulations in the previous guinea pig studies, in which the Carbomer Cream and the Glycerol Cream were the most well-tolerated formulations.

In humans, the IPP Cream, known to be the least tolerable formulation in guinea pigs was the most detrimental treatment. The Basic Cream, which was also in-tolerable to guinea pigs, was in humans similar to 'Not treating', but better than IPP cream. This difference in ranking was not found in earlier studies in which neither species differentiated between these two formulations.

The ranking of the carbomer cream was essentially the same in the two species, the main difference being that this cream in humans was similar to 'No Treatment' rather than worse. The results show that both change of emulgator system and addition of anti-irritants may have an influence on the properties of skin care formulations in relation to cumulative skin irritation.

The addition of glycerol to the carbomer cream improved the performance, while adding (–)-α-bisabolol or canola oil had no anti-irritant effect in acute irritation studies. This finding was repeated in the current cumulative irritation study in which the only treatment clearly better than 'No Treatment' was the Glycerol Cream. The choice of vehicle and concentrations may affect the result but we saw no beneficial effect on the cutaneous irritation induced by SLS and NON, respectively.

In summary, the results from the hairless guinea pig study and the human volunteers study were in agreement with regard to ranking of the skin care formulations (Table 4). However, a positive treatment effect of the Glycerol Cream on both SLS and NON irritated skin in human volunteers was found in comparison to 'No Treatment'. The Basic cream was better tolerated in humans than was expected from previous testing in hairless guinea pigs.

3.3.3 The hairless guinea pig as a cutaneous model, conclusions

The hairless guinea pig may be used as a sensitive tolerability model and a model for cumulative irritation. The acute guinea pig model is handicapped by the rapid natural healing and should not be used. Formulations found in the guinea pig models to be well-tolerated and with treatment effect better as or similar to "not treatment" are likely to be effective treatments in human models.

The unexpected finding that glycerol, intended to be used as a humectant, apparently was a more effective anti-irritant than both canola oil and (–)-α-bisabolol in both guinea pig and human models made us include glycerol as a potential anti-irritant in the last three studies focusing on demonstrating anti-irritant effect of selected published anti-irritants in humans.

3.4 ANTI-IRRITANTS: EVALUATION OF CONCEPT

The rationale behind the following studies was to develop a three-step method for the evaluation of effect of alleged anti-irritants in the treatment of experimental irritation caused by two complementary irritants, SLS and NON.

The four alleged anti-irritants: nifedipine, (–)-α-bisabolol, canola oil and glycerol were formulated in the vehicle Basis

Table 4. Effect of skin care formulations on cumulative irritation in hairless guinea pigs and volunteers

	Clinical Score AUC		Colorimetry a*-parameter		TEWL g/m²h		Hydration µS	
Efficacy	best	worst	best	worst	best	worst	best	worst
HLGP	0 G P B C		0 G B P C		0 G P B C		Not measured	
HV SLS	G F 0 B P C I		G F 0 B P I C		G F 0 B P I C		G P B 0 C F I	
HV NON	G 0 F B P C I		G 0 F B P C I		G F 0 B P C I		G F 0 P B C I	

Underlining of two SCF means, that they are not significantly different. An Example:
The Clinical Score (AUC) results for HLGP (0 G P B C) can be interpreted as follows: The order 0 G P B C indicates that the level of response to No treatment was less than that to G, whilst the response to G was less than that to P etc. The underlining of P and B however, indicates that with statistical testing, the differences in response level seen between P and B were not statistically significant. Therefore, the statistical ranking in terms of which formulation is the most effective treatment is, as assessed by AUC in HLGP: 1st-0; 2nd G; 3rd P and B; 4th -C.
0 denotes No treatment, F basic cream, P carbomer cream, I IPP cream, G glycerol cream, C canola oil cream, B bisabolol cream, HLGP hairless guinea pigs, HV human volunteers. F and I were not tested in HLGP due to low tolerability

Salve, an ointment consisting of 10% polyethylene and 90% paraffin oil.

The fifth study focused on dose-related effect of the treatments in an acute irritation model to select the most effective anti-irritant concentration. In the sixth study the 4 anti-irritants at optimum concentrations were to be compared in a cumulative irritation model. In the seventh and final study the best anti-irritant was compared with a commonly used topical group II corticosteroid.

3.4.1 Dose-response in an acute irritation model (Paper V)

Sample sizes and irritant concentration were chosen based on a pilot study. Acute irritation was induced by 24h occlusive tests with 1% sodium lauryl sulfate aq. (SLS) on the right and 20% nonanoic acid in n-propanol (NON) on the left volar forearm of healthy volunteers. Eight patches were applied on each volar forearm of the subjects (Fig. 7A).The irritant reactions were treated twice daily with the formulations from the time of

Table 5: Hydration levels in sites either left untreated or treated with glycerol [a)]

	1% Sodium Lauryl Sulphate (SLS)	20% Nonanoic Acid (NON)
DAY 4		
No Treatment (0)	55.0 (46.7-71.7)	50.0 (28.3-63.3)
5% Glycerol (G_5)	61.7 (46.7-81.7)	51.7 (30.0-73.3)
10% Glycerol (G_{10})	65.0 (50.0-86.7)	46.7 (36.7-70.0)
20% Glycerol (G_{20})	71.7 (53.3-93.3)	58.3 (35.0-73.3)
EOT		
No Treatment (0)	46.7 (36.7-68.3)	51.7 (43.3-60.0)
5% Glycerol (G_5)	46.7 (40.0-63.3)	60.0 (38.3-76.7)
10% Glycerol (G_{10})	60.0 (43.3-76.7)	66.7 (51.7-81.7)
20% Glycerol (G_{20})	65.0 (56.7-100.0)	70.0 (46.7-86.7)

[a)] Data are given as median (interquartile range). Units are µS.
EOT: End of Treatment (Day 7).

removal of the patches. Anti-irritant concentrations were chosen based on a literature.

With 8 patch test sites and 8 treatment modalities (2 anti-irritants in 3 concentrations, vehicle and 'No Treatment'), treatments could be randomized to sites using a Latin Square Design. The anti-irritants were studied in two groups: in the first group nifedipine and canola oil were studied, in the second group (–)-α-bisabolol and glycerol were studied as treatments for acute irritation.

Reactions were evaluated clinically and by noninvasive methods on Days 1–4 and at End of Treatment on Day 7.

Only glycerol showed any improvement in healing rate when compared to 'No Treatment', glycerol, also dose-response relationship was seen only for glycerol and only in regard to cutaneous hydration (Table 5). As glycerol is a well-known humectant the result is not too surprising.

Statistical ranking for the two groups of anti-irritants are shown in Tables 6 and 7. The random ordering of treatment effects of the other anti-irritants suggests that the lack of dose-response was not due to too small a sample size.

The findings in the current study are in agreement with our studies of the effect of skin care formulations on acute and cumulative irritation in hairless guinea pigs and humans in which only glycerol, but not canola oil and (–)-α-bisabolol, exhibited positive effect (Papers III and IV).

The lack of effect could be attributed to the small window of opportunity in which to demonstrate efficacy, as the natural healing of an acute irritant response is very fast.

In conclusion, the acute irritation model gives no support for the use anti-irritants as treatments of cutaneous irritation.

3.4.2 Comparison of anti-irritants in a cumulative irritation model (Paper VI)

As no dose-related treatment effect was found in the acute model, except for glycerol, the highest concentration of each of the 4 anti-irritants was chosen as a treatment modality in the cumulative model, as this would give a larger window of opportunity in which to study the effect of the treatments.

The cumulative irritation model described in paper IV was used, inducing irritant dermatitis with 10 minutes daily exposures for 5 + 4 days (no irritation on weekend) to the irritants. To avoid too many axaggerated responses the irritant concentrations were this time 1% SLS on the right and 20% nonanoic acid (NON) on the left volar forearm (Fig. 7B). Anti-irritant ointments were applied twice daily. Clinical scoring was performed daily, evaporimetry (TEWL), hydration and colorimetry were measured at baseline (day 0) in the middle and at the end of treatment.

The reaction patterns to the two irritants (Table 8) are in accordance with previous studies (Paper IV).

In Fig.e 5 depicting clinical irritation is seen a flattening of the curve on days 5 and 6 followed by an increase from day 7 on is due to cessation of irritation in the weekend. Thus, the model emulates the pattern seen in occupational contact dermatitis, i.e. a reduction in the symptoms on weekends and holidays, followed by an increase in symptoms when work is resumed.

Apparently 20% NON was better suited than 1% SLS for separating the effect of the treatments, emphasizing the importance of not relying on one model irritant for cutaneous irritation studies.

As shown in Table 9 all the treatments were, by at least one assessment, found to be better than Not Treating; but only glycerol ointment was better than vehicle in concordance with the acute irritation study (Paper V).

Table 6. Statistical ranking of nifedipine and canola oil in the acute model.

	Clinical Score		Colorimetry a*-parameter		TEWL g/m²h		Hydration µS	
Efficacy	best	worst	best	worst	best	worst	best	worst
SLS	$N_{0.25}N_1N_{0.5}$ 0 $C_{20}C_{40}$ V C_{10}		C_{10} $N_{0.25}$V 0 $N_{0.5}$ N_1 C_{40} C_{20}		$C_{10}N_1N_{0.25}N_{0.5}VC_{20}C_{40}$ 0		$N_{0.25}$ $N_1N_{0.5}$ $VC_{10}C_{40}0C_{20}$	
NON	N_1 C_{40} V $C_{10}N_{0.25}$ C_{20} $N_{0.5}$ 0		C_{20} N_1 $N_{0.5}$ C_{10} V $N_{0.25}$ 0 C_{40}		V $N_1C_{20}N_{0.5}C_{10}N_{0.25}C_{40}0$		$C_{40}N_{0.5}C_{20}C_{10}N_{0.25}VN_10$	

Underlining of two anti-irritants means that they are not significantly different.

For Example: The Clinical Score for SLS can be interpreted as follows: The order $N_{0.25}N_1N_{0.5}$ 0 $C_{20}C_{40}$ V C_{10} indicates that the observed effect of V was better than C_{10}, whilst the observed effect of C_{40} was better than V etc. The continuous underlining indicates that none of the observed differences were statistically significant.

SLS: 1% sodium lauryl sulphate; NON: 20% nonanoic acid;
0: no treatment; V: vehicle (basis salve);
$N_{0.25}$: 0.25% nifedipine; $N_{0.5}$: 0.5% nifedipine; N_1: 1.0% nifedipine;
C_{10}: 10% canola oil; C_{20}: 20% canola oil; C_{40}: 40% canola oil.

Table 7. Statistical ranking of glycerol and (-)-α-bisabolol in the acute model.

Efficacy	Clinical Score best	Clinical Score worst	Colorimetry a*-parameter best	Colorimetry a*-parameter worst	TEWL g/m²h best	TEWL g/m²h worst	Hydration µS best	Hydration µS worst
SLS	$G_5B_{0.5}$ $B_{0.1}$ V $B_{0.25}G_{20}G_{10}$ 0		$G_5G_{10}G_{20}B_{0.5}$ V $B_{0.1}$ 0 $B_{0.25}$		$G_5G_{10}G_{20}B_{0.5}B_{0.25}VB_{0.1}0$		G_{20} G_{10} $B_{0.1}G_5B_{0.5}$V 0$B_{0.25}$	
NON	0 $G_5VB_{0.25}$ $B_{0.1}$ $B_{0.5}$ $G_{10}G_{20}$		$B_{0.25}G_5$ $B_{0.5}$ G_{20} 0 $B_{0.1}$ G_{10}V		$G_5B_{0.5}VB_{0.25}G_{10}G_{20}B_{0.1}0$		$G_{20}G_{10}$ G_5 V $B_{0.1}B_{0.5}B_{0.25}$0	

Underlining of two anti-irritants (AI) means that they are not significantly different.

For Example: The Hydration results for NON can be interpreted as follows: The order $G_{20}G_{10}G_5$ V $B_{0.1}B_{0.5}B_{0.25}$ 0 indicates that the observed effect of $B_{0.25}$ was better than 0, whilst the observed effect of $B_{0.5}$ was better than $B_{0.25}$ etc. The underlining of G_{20} and G_{10} indicates that, the observed difference in effect level seen between G_{20} and G_{10} was not statistically significant. However the observed differences seen between G_{20} and all other AI were statistically significant. Likewise, the observed differences between G_{10} and G_5 and V were not statistically significant, whereas the observed differences between G_{10} and all bisabolol-concentrations (B) as well as 0 were. In other words, there is some support for a dose-response effect of glycerol (G) in relation to not treating, whereas bisabolol has no apparent effect.

SLS: 1% sodium lauryl sulphate; NON: 20% nonanoic acid;
0: no treatment; V: vehicle (basis salve);
G_5: 5% glycerol; G_{10}: 10% glycerol; G_{20}: 20% glycerol;
$B_{0.1}$: 0.1% bisabolol; $B_{0.25}$: 0.25% bisabolol; $B_{0.5}$: 0.5% bisabolol.

Using a carbomer cream as vehicle as well as different concentrations of (–)-α-bisabolol, glycerol and canola oil the same outcome was found in studies of the hairless guinea pig as a model for acute and cumulative irritancy (Papers III and IV).

Even with different vehicles and range of concentrations (–)-α-bisabolol and canola oil were without any effect.

Only 1 of 4 alleged anti-irritants i.e. glycerol, demonstrated treatment effect better than No Treatment and vehicle against cutaneous irritation. Thus new chemicals with potential anti-irritant effect should be tested in human models mimicking the natural course of contact dermatitis.

3.4.3 Comparison of glycerol with a group II corticosteroid (Paper VII)

Finally glycerol was benchmarked against a commonly used group II corticosteroid, Kenalog® (triamcinolone acetonide) formulated in an ointment base similar to basis salve, the vehicle used in the anti-irritant studies.

Forearm wash tests are commonly used to compare mildness of detergents (90–93). Comparing wash tests, patch test and repeated open applications of a detergent it was found that the three methods do not necessarily correlate, as they affect the cutaneous barrier in different ways. The forearm wash tests appear to be even more realistic than repeated open applications of irritants (94–96).

Table 8. Baseline and End of Treatment biometric values in untreated cumulative irritation[a]

Biometric Measurement		1% SLS, 5+4 days	20% NON, 5+4 days
Colorimetry a*parameter	Baseline	7.3 (6.4-8.5)	7.8 (6.8-8.8)
	Day 4	7.2 (6.4-8.5)	7.4 (6.5-7.9)
	EOT	7.2 (5.9-8.8)	6.8 (6.1-8.1)
TEWL g/m²h	Baseline	7.9 (6.5-8.9)	7.6 (6.5-8.5)
	Day 4	11.2 (8.8-12.5)	9.2 (8.1-10.8)
	EOT	14.0 (11.7-16.7)	11.3 (9.9-13.0)
Hydration µS	Baseline	53.3 (45.0-61.7)	53.3 (46.7-65.0)
	Day 4	40.0 (30.0-48.3)	30.0 (23.3-38.3)
	EOT	40.0 (30.0-55.0)	30.0 (26.7-45.0)

[a] Data are given as median (interquartile range). EOT End of treatment (Day 11).
SLS: Sodium Lauryl Sulfate. NON: Nonanoic Acid.

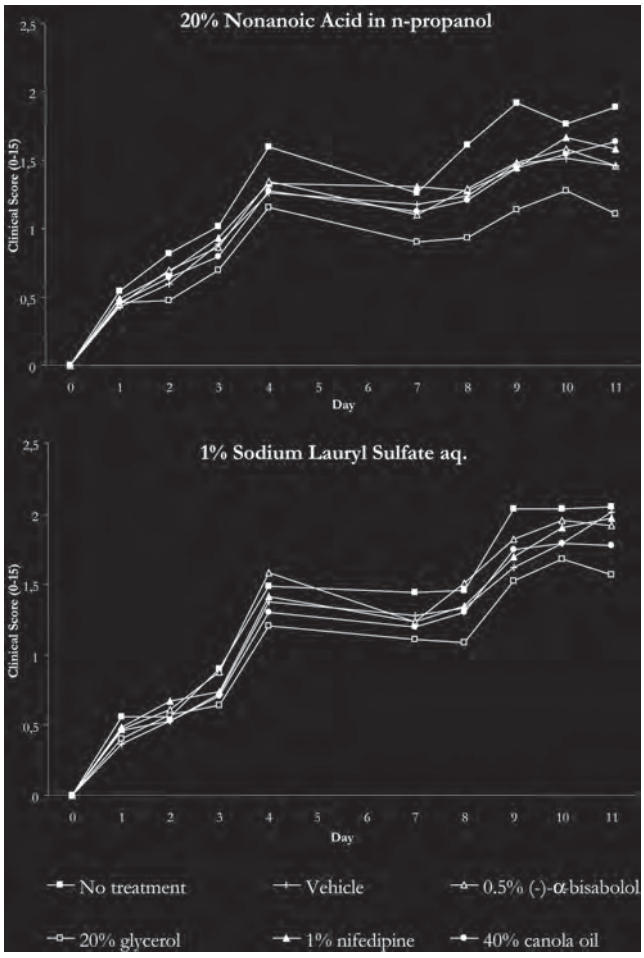

Fig. 5. Development of cutaneous irritation assessed clinically from Baseline to End of Treatment. Data are given as means.

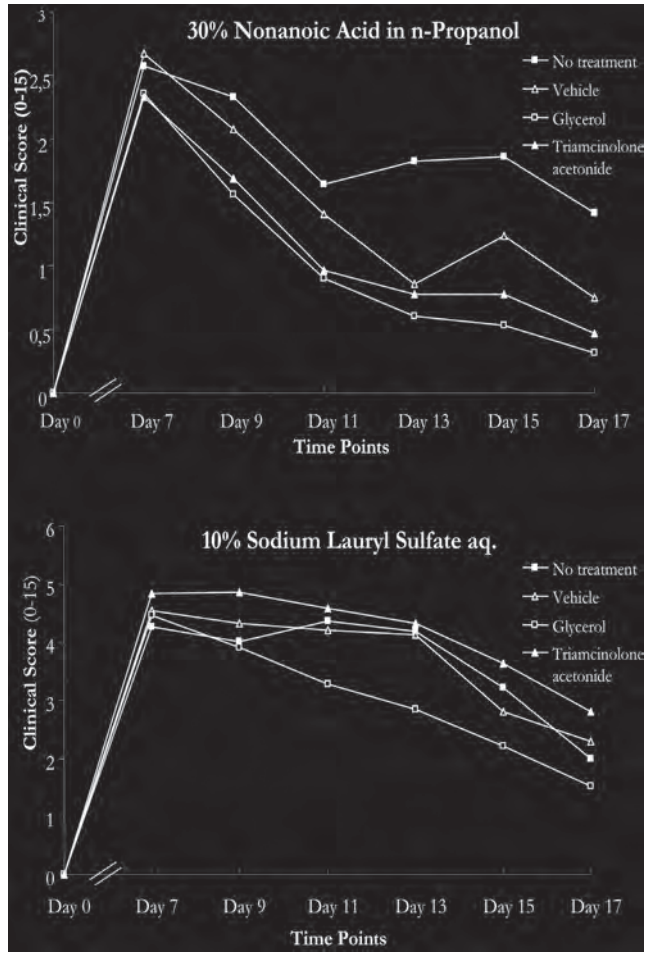

Fig. 6. Development of cutaneous irritation assessed clinically from Day 0 to Day 17 (End of Treatment). Data are given as means.

For comparing glycerol with the corticosteroid a human forearm wash test was developed intended to mimic every day life, in which the patient first develops a contact dermatitis, then consults the physician and is told to reduce/avoid irritant exposure and is given a treatment.

On each volar forearm 4 sites were marked and baseline readings obtained on Day 0. A state of cumulative contact dermatitis was provoked by washing thrice daily for a week with SLS 10% aq. on the right and NON 30% in n-propanol on the left volar forearm (Fig.e 7C). The aim was to obtain a total clinical score of 2, a TEWL of at least 15g/m^{2}h for SLS, and a hydration level at least as low as 30 ¼S for NON corresponding to a moderate reaction.

Following assessment of the degree of irritation reached on Day 7, the volunteers were told to reduce the number of daily washings to 2 or less to maintain a suitable level of irritation. Twice daily following washings volunteers, following a latin square, treated the 4 sites on each arm with glycerol, corticosteroid, vehicle and "No treatment" in a double-blinded fashion. Sites were assessed every other day for a two week period.

The development of cutaneous irritation from Day 0 to Day 17 is shown in Fig. 6. Statistical ranking of treatments is shown in Table 10.

For NON-irritation treatments were ranked similarly whether assessed clinically or by any biometric method. Both glycerol and triamcinolone acetonide, were statistically significantly better than not treating. Vehicle was clinically better than not treating but this difference was not statistically significant. Triamcinolone acetonide produced an effect which lay between that of glycerol and vehicle but statistically could not be distinguished from either.

For SLS-irritation treatments were ranked similarly whether assessed clinically or by hydration. Vehicle was not found to have an effect. Triamcinolone acetonide significantly worsened the irritation. Glycerol reduced the irritation though the improvement was not statistically significant. The statistically significant differences found using the Mixed Models were also found using non-parametric Wilcoxon Signed Rank Tests.

Comparing glycerol and a corticosteroid, differences between treatments were clear. Glycerol is barrier stabilizing and hydrating (62;63;68–70), where as the corticosteroid is an anti-inflammatory agent with negative effect on the stratum corneum barrier (3;97;98). Thus glycerol induced faster healing of both the SLS and NON induced irritation, whereas the corticosteroid induced faster healing of the NON-induced irritation but worsened the SLS-reaction, indicating further barrier disruption.

These findings are supported by the finding of Anveden and co-workers that oral prednisone has no effect on patch test reactivity to SLS and NON, raising concern about uncritical use

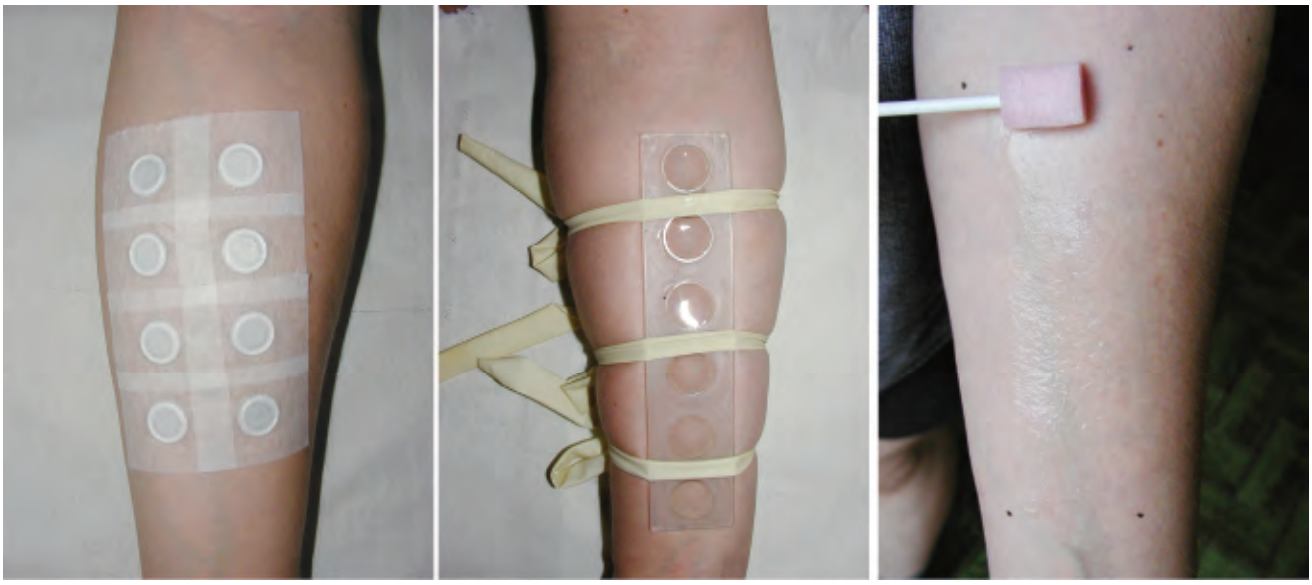

Fig. 7. A. Acute model. B. Cumulative model. C. Wash test

Table 9. Statistical ranking of anti-irritants as treatments of cumulative irritation.

	Clinical Score AUC		Colorimetry a*-parameter		TEWL g/m^2h		Hydration µS	
Efficacy	best	worst	best	worst	best	worst	best	worst
1% SLS	G V C B O N		G O B C V N		G B O N C V		G V B C N O	
20% NON	G C V B N O		G V C O B N		G B N C V O		G N B C V O	

Underlining of two AI means, that they are not significantly different.

For Example: The Clinical Score results for NON can be interpreted as follows: The order G C V B N O indicates that the observed effect of AI N was better than AI O, whilst the effect of AI B was better than AI N etc. The underlining of C, V, B and N and of N and O indicates that with statistical testing, neither the observed difference in effect seen between AI C, V, B and N, nor the observed difference between AI N and O were statistically significant. Therefore, the statistical ranking in terms of which formulation is the most effective treatment, as assessed by Clinical Score in irritation induced by NON, is that AI G is clearly better than either C, V, B, N and O, whilst O is clearly worse than G, C, V and B, but that N could be as good as C, V, B or as poor as O.

AI denotes anti-irritants, 0 denotes no treatment, V vehicle, B (-)-α-bisabolol ointment, C canola oil ointment, G glycerol ointment and N nifedipine ointment. SLS: Sodium Lauryl Sulfate. NON: Nonanoic Acid.

of corticosteroids as treatments for irritant contact dermatitis (99).

In summary glycerol appears to be an effective anti-irritant as well as a humectant.

3.4.4 Anti-Irritants: Evaluation of concept, conclusions

The models described herein were designed as a tool for proof of concept for potential anti-irritants. By dimensioning the studies to enable detection of a relatively large effect difference (3.5 g/m^2h), only potent anti-irritants were identified. By increasing the sample-size, more anti-irritants (weaker) might be detected in future studies.

Glycerol was the only compound, in a series of studies compared to four alleged anti-irritants, showing beneficial effect on experimental irritation to two complementary irritants demonstrates the need for testing of alleged anti-irritants prior to their incorporation in formulations.

Efficacy	Clinical Score best	Clinical Score worst	Colorimetry a*-parameter best	Colorimetry a*-parameter worst	TEWL g/m^2h best	TEWL g/m^2h worst	Hydration µS best	Hydration µS worst
SLS	G 0 V T		T G V 0		G V T 0		G 0 V T	
NON	G T V 0		T G V 0		G T V 0		G V T 0	

Underlining of two treatments means that they are not significantly different.
For Example: The hydration results for SLS can be interpreted as follows: The order G 0 V T indicates that the observed effect of V was better than T, whilst the observed effect of 0 was better than V etc. The underlining of 0 and V indicates that with statistical testing the observed difference in effect level seen between 0 and V was not statistically significant. Therefore, the statistical ranking in terms of which formulation is the most effective treatment, as assessed by hydration, is that G is clearly better than 0, V and K; 0 and V are clearly better than T;. but there is no difference between V and 0.
0 denotes no treatment, V vehicle, G glycerol ointment and T Triamcinolone acetonide ointment.

4. OVERALL CONCLUSIONS AND PERSPECTIVES FOR THE FUTURE

Corticosteroids should not be used uncritically as treatments for irritant contact dermatitis.

Anti-irritants intended to be used as treatment modalities should exhibit dose-related effect demonstrated in human models simulating contact dermatitis. Otherwise they are only to be considered "window dressing".

The herein described methods should be further developed and validated. As the acute model is both unrealistic and heals too fast it should be replaced by a cumulative model, to provide more insight in dose-related effectiveness of the new chemical entities.

The differences in treatment efficacy of glycerol and corticosteroid on irritation induced by the complementary irritants SLS and NON should be exploited further by developing the model further, so that both glycerol and the corticosteroid are used as positive treatment controls.

A modified version of the three-step model developed could in the future be used in proof of concept studies prior to larger clinical studies.

5. SUMMARIES

5.1. ENGLISH SUMMARY

Allergic and irritant contact dermatitis (CD) is a common problem in environmental medicine. Irritant CD is the most common entity and is typically induced by wet work, dirt and detergents. CD is often localized to the hands and often leads to sick notes, change of job and even revalidation, being a burden for both the individual and the society.

The aim of the project was to study the effect of so called anti-irritant (AI) substances as treatments for experimentally induced contact dermatitis. AI are defined as substances which, when used in conjunction with skin or eye irritants, reduces their irritation potential sufficiently to be tolerated when applied to the body.

Based on literature studies the following four anti-irritants were chosen, as they were found safe and reasonably well-documented.

- Nifedipine is a calcium channel blocker, used in cardiology. Anti-irritative effect has been demonstrated in vitro and in mouse studies.
- (–)-α-bisabolol is the most active stereo isomer of bisabolol from chamomille oil. Inhibits the enzymes 5-lipoxygenase and cycloxygenase of the arachidonic acid cascade. Effect has been demonstrated in in vitro, animal and human studies.
- Canola oil, a low erucic acid level Canadian rape seed oil. Effect has been demonstrated in a human model. Lipid donor.
- Glycerol, commonly used as humectant in vehicles and cosmetic formulations. Demonstrated effect in the first series of studies and was included in the final studies as a potential AI.

The project consisted of two phases. In the first phase the hairless guinea pig (HLGP) was studied as a model for cutaneous tolerability of new topical formulations and as model for the treatment of experimentally induced acute and cumulative irritation and compared with similar studies in healthy human volunteers. Six composite topical formulations were selected as model vehicles. The skin care formulations, with and without either isopropyl palmitate, glycerol, canola oil or (–)-a-bisabolol, were selected because they were known to cause a differentiated irritative response in HLGP. The ranking of the formulations was similar in the two models, but the HLGP were more sensitive than human skin. In conclusion HLGP may be used as a screening tool for new formulations; however negative results in the HLGP do not necessarily indicate that a topical formulation cannot be used in humans.

In the second phase, the four model anti-irritants all in a common vehicle (basissalve) were tested in a series of three studies in human volunteers as treatment for experimentally induced acute and cumulative cutaneous irritation. The same model irritants were chosen: SLS, known to cause barrier damage with minor inflammation, and NON, causing severe inflammation with minimal barrier damage.

The first study aimed to demonstrate dose-response of AI by treating acutely irritated skin with three concentrations of each of the 4 AI. The best concentrations of each of the 4 AI were then compared in an open cumulative irritation assay, in order to determine the best AI. Finally the most effective AI was compared to a group II corticosteroid in a skin wash model.

In the first study, only glycerol showed potential dose-response and positive effect. For the second study the strongest AI concentrations were chosen, again only glycerol showed consistent effect, and was chosen as model AI for the last study. In this we found that glycerol and corticosteroid were equally effective as treatments for irritation induced by NON, where as glycerol was the only effective treatment for SLS-induced irritation, corticosteroid-treatment was detrimental to the restoration of the cutaneous barrier.

In conclusion working models for efficacy testing of new treatment modalities such as anti-irritants has been developed.

5.2 DANSK RESUMÉ

Allergisk og irritativ kontaktdermatitis er et almindeligt problem i arbejdsmedicin. Irritativ kontaktdermatitis forekommer hyppigst og induceres typisk af vådt arbejde, snavs og rengøringsmidler. CD findes ofte på hænderne og fører ofte til sygefravær, jobskifte og sågar revalidering, og er derved en byrde både for den enkelte og for samfundet.

Målet med projektet var at studere virkningen af såkaldte anti-irritanter som behandlings modaliteter for eksperimentelt induceret kontakt dermatitis. Anti-irritanter defineres som stoffer, der når de anvendes sammen med hud- eller øjenirritanter dæmper irritationen fra disse i en sådan grad at de kan tolereres på kroppen.

De følgende fire anti-irritanter blev valgt på basis af litteraturstudier fordi de dels var sikre og rimeligt veldokumenterede.

- ifedipine er en calcium kanal blokker, der bruges i kardiologi. Anti-irritativ effekt er demonstreret i in-vitro og musestudier. Virkningsmekanismen er ikke kendt til bunds.
- (–)-α-bisabolol er den mest active stereo-isomer af bisabolol, der udvindes af kamille olie. Virker ved at hæmme enzymerne 5-lipoxygenase og cycloxygenase i arachidon syre kaskaden. Effekt er blevet vist in-vitro, i dyr og i humane studier.
- Canola oil, er en canadisk rapsolie med et lavt indhold af erucin syre. Effekt er vist i en human model. Virker antagelig som lipid donor.
- Glycerol, almindeligt anvendt humectant i farmaceutiske vehikler og kosmetiske formuleringer. Var medtaget som humectant i et vehikel i den første serie studier, viste anti-irritativ effekt og blev medtaget i de sidste tre studier.

Projektet bestod af to dele. I den første del blev det hårløse marsvin vurderet som model for kutan tolerabilitet og som model for heling af akut og kumulativ kontakt dermatitis. Resultaterne blev sammenlignet med resultaterne fra tilsvarende forsøg i frivillige forsøgspersoner. Som forsøgsstoffer anvendtes 6 modelvehikler. Disse hudplejeformuleringer, hvoraf nogle indeholdt isopropyl palmitate, glycerol, canola oil eller (–)-a-bisabolol, blev valgt fordi de var kendt for at give et varieret hudrespons i det hårløse marsvin. Marsvin og menensker rang-

ordnede vehiklerne på tilsvarende vis i modellerne, men marsvinene var mere følsomme end de frivillige forsøgspersoner. Vi konkluderede at det hårløse marsvin kan bruges som et redskab til screening af nye formuleringer. Men at marsvin ikke tolererer en formulering er ikke ensbetydende med at mennesker ikke kan anvende den.

I den anden del blev de fire anti-irritanter i et fælles vehikel (basissalve) studeret i tre forsøg med frivillige forsøgspersoner som behandlingsmodaliteter for akut og kumulativ kontaktdermatitis. I alle studier blev de samme irritanter anvendt, en korrosiv irritant natrium lauryl sulfat og en ikke-korrosiv irritant, nonansyre.

I det første af disse forsøg var målet at demonstrere dosis-respons ved at anvende tre koncentrationer af hver af de fire anti-irritanter som behandlinger for akut irritation. Den bedste/højeste koncentration af hver anti-irritant blev derefter sammenlignet i en kumulativ irritations model, for at bestemme den bedste anti-irritant. Endelig blev behandlingseffekten af den mest effektive anti-irritant sammenlignet med et gruppe II kortikosteroid's i en hudvaskemodel.

I det første studie viste kun glycerol tilnærmelsesvis dosis-respons og behandlingseffekt. I det andet studie blev den højeste koncentration af anti-irritanterne anvendt, her viste kun glycerol sikker behandlingseffekt. I det sidste studie viste det sig at glycerol og kortikosteroid var ligeværdige behandlingsmodaliteter for nonansyre induceret irritation, medens kun glycerol udviste sikker behandlingseffekt overfor SLS-induceret irritation. Kortikosteroid hæmmede tilsyneladende ophelingen.

Sammenfattende er der blevet udviklet modeller, der kan bruges til "proof of concept" studier for nye behandlingsmodaliteter såsom anti-irritanter.

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