

Evaluating skin-protective materials against contact irritants and allergens

An in vivo screening human model

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2 acute irritants and 1 allergen were selected: sodium lauryl sulfate (SLS) representative of irritant household and occupational contact dermatitis, the combination of ammonium hydroxide (NH₄OH) and urea to simulate diaper dermatitis, and *Rhus* to evaluate the effect of model protective materials. The putative protective materials and vehicle were applied to both ventral forearms of 10 subjects in each group, according to a randomized code. Test materials were spread over a marked 2.0 cm² area, massaged in, allowed to dry for 30 min, and reapplied with another 30 min drying period. The model irritants and allergen were then applied (0.025 ml) to an AI-test[®] occlusive patch, which in turn was placed for 24 h over each of the 8 designated sites. Inflammation was scored according to a clinical scale 72 h post-application. Paraffin wax plus Acetulan[®] in cetyl alcohol, and beeswax plus Acetulan[®] in cetyl alcohol, markedly ($p < 0.001$) suppressed SLS irritation. Paraffin wax plus beeswax in cetyl alcohol, and Acetulan[®] in cetyl alcohol reduced NH₄OH and urea irritation ($p < 0.05$), paraffin wax in cetyl alcohol significantly ($p < 0.01$) decreasing *Rhus* allergic contact dermatitis. This model, provides an easy approach to screening protectants. Its clinical significance requires comparison with an open rather than an occluded challenge.

Key words: protection materials; irritation; sodium lauryl sulfate (SLS); urea; *Rhus*; ammonium hydroxide (NH₄OH); contact dermatitis (CD). © Munksgaard, 1998.

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Barrier creams (BC) against irritant and allergic contact dermatitis, have been alleged to provide benefit (1): several techniques to assess barrier creams, efficacy have been reported (1–10). Some may not be suitable for screening, because of their complexity and time-consuming nature (6, 7, 9, 10). Animal experiments may not be uniformly extrapolatable to humans (2, 3, 6, 9, 10).

For screening purposes, we utilized an in vivo human model to examine the effect of potential protective model materials against 2 acute irritants (SLS, and NH₄OH plus urea) and 1 allergen (*Rhus*) that model clinically relevant situations.

Materials and Methods

Subjects

10 healthy, male Caucasian volunteers (mean age 41.2 ± 5.8 years) participated after providing informed consent. The study was approved by the University of California, San Francisco, Human Research Committee.

Irritants

Group 1: 10.0% sodium lauryl sulfate (SLS) (99% purity, Sigma Chemical Co., St. Louis, MO) in water.

Group 2: 22.9% ammonium hydroxide (Fisher Scientific, Fair Lawn, NJ) and 2.0% urea (Ashland Chemical Co., Columbus, OH) in water.

Group 3: 3.0% *Rhus* allergen (Hollister-Stier, Spokane, WA) in acetone.

Test materials and preparations

All test materials were purchased commercially. The materials selected for the test protective formulations were selected because of their alleged protective properties. Beeswax and paraffin wax were selected for their emollient dermal interaction and barrier properties to water-soluble materials. Acetulan[®], a lanolin derivative, imparts hydrophobic properties to the skin. Cetyl alcohol was selected as a solvent because it is lipophilic toward

Table 1. Patch test grading scale

0	none
1	barely visible erythema
2	weak spotty without itching
3	weak spotty with itching, but without edema
4	diffuse erythema with weak edema, involving less than 25% of the test site
5	as in 4, plus moderate edema, involving 25% to 50% of the test site
6	as in 5, plus severe edema and scattered vesicles, involving 50% to 75% of the test site
7	as in 6, plus vesicles confluent, involving more than 75% but limited in a circular pattern on the test site
8	as in 7, plus bullae, or weak erosions
9	erythema, edema, vesiculation, plus evidence of severe ulcerative breakdown

the lipid barrier forming materials, and adds to the penetrating and spreading properties of other formulation ingredients.

The experimental preparations and test sites were designated as below:

- (a) PW+CA: paraffin wax (PW) in cetyl alcohol (CA);
- (b) PW+A+CA: paraffin wax (PW) plus Acetulan® (A) in cetyl alcohol (CA);
- (c) PW+B+CA: paraffin wax (PW) plus beeswax (B) in cetyl alcohol (CA);
- (d) B+CA: beeswax (B) in cetyl alcohol (CA);
- (e) B+A+CA: beeswax (B) plus Acetulan® (A) in cetyl alcohol (CA);
- (f) A+CA: Acetulan® (A) in cetyl alcohol (CA);
- (g) CA: cetyl alcohol (CA);
- (h) U: untreated skin

Methods

Test materials were applied to all subjects in each group according to a randomized code; each forearm had 4 premarked sites. A spatula was used to apply each protectant or vehicle to the site. 1 site served as an untreated control, and received the irritant or allergen only. Each test material (0.05 ml) was spread over a 2.0 cm² area, massaged in, allowed to dry for 30 min, and reapplied with another 30 min drying period. The appropriate irritants or allergen were then applied (0.025 ml) to an Al-test® (IMECO AB, Södertälje, Sweden) occlusive patch, which in turn was placed over each of the designated sites. The patches were secured with plastic (Blenderm®, 3M, St. Paul, MN) surgical tape, and overlapped with 3 in paper (Micropore®, 3M, St. Paul, MN) surgical tape to assure continuous patch contact. The patches were removed after 24 h. Inflammation was scored according to an expanded scale, shown below, 72 h post-application. This single point observation was the same for all subjects in order to permit ranking. The lower the aver-

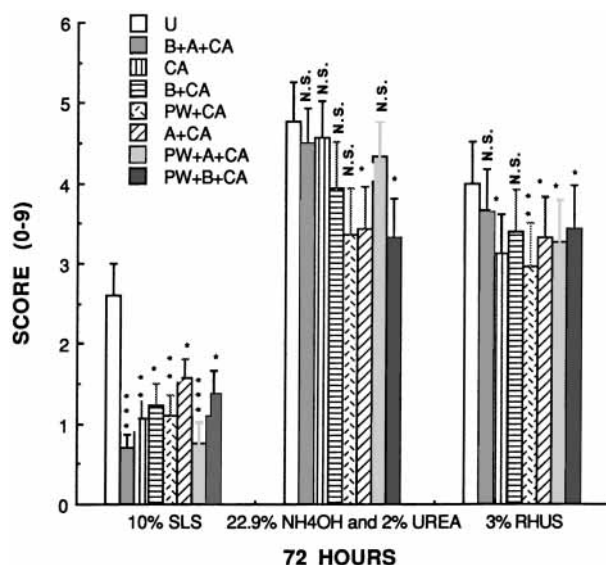


Fig. 1. Efficacy of protective materials against skin irritation following treatment by 2 acute irritants (10% SLS, 22.9% NH₄OH and 2% urea) and 1 allergen (3% *Rhus*). Results expressed as the means±SE. Statistical differences in comparison with untreated skin site. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS: not significant.

age score, the more effective the treatment. The *Rhus* panel was positive by history and prescreening (patch test) to the same allergen.

Clinical scale

Cutaneous reactions were scored a 10 point scale (Table 1). This expanded scale, with appropriate training, provides greater discrimination than narrow scales.

Statistics

Wilcoxon signed rank test was performed for the nonparametric score. The sign test evaluated differences within paired scores based solely on whether 1 score is larger or smaller than the other. This test examines both the direction and the relative amount of difference. Friedman test was performed for analysis of variance by ranks of all test sites.

Results

Group 1: SLS

All test materials showed a statistically significant decrease in SLS irritation in comparison with the untreated control site (Fig. 1): paraffin wax plus Acetulan® in cetyl alcohol and beeswax plus Acetulan® in cetyl alcohol markedly ($p<0.001$) suppressed irritation.

Table 2. Rankings of materials by effectiveness for Groups 1, 2 and 3

Protective materials	Average rank	Group 1 (SLS)	Group 2 (NH ₄ OH and urea)	Group 3 (<i>Rhus</i>)
paraffin wax, cetyl alcohol	4.04	4.12**	4.08 (NS)	3.93**
paraffin wax, acetulan [®] , cetyl alcohol	4.18	3.55***	4.68 (NS)	4.30*
paraffin wax, beeswax, cetyl alcohol	4.33	4.45*	3.87*	4.67*
cetyl alcohol beeswax, cetyl alcohol	4.38	4.47**	4.93 (NS)	3.73*
cetyl alcohol beeswax, acetulan [®] , cetyl alcohol	4.41	4.27*	4.43 (NS)	4.52 (NS)
acetulan [®] , cetyl alcohol	4.47	3.83***	4.57 (NS)	5.02 (NS)
cetyl alcohol untreated skin	4.48	5.30*	4.02*	4.12*
	5.70	6.02	5.42	5.67

Statistical differences in comparison with untreated skin site. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS: not significant

Group 2: ammonium hydroxide (NH₄OH) plus urea

Only paraffin wax plus beeswax in cetyl alcohol and Acetulan[®] in cetyl alcohol revealed a significant ($p < 0.05$) reduction in irritation.

Group 3: *Rhus*

Most test materials, except the beeswax in cetyl alcohol, and beeswax plus Acetulan[®] in cetyl alcohol, statistically suppressed the allergic reaction to *Rhus*. Paraffin wax in cetyl alcohol was the most effective ($p < 0.01$) in decreasing dermatitis.

Ranking of effectiveness

Each test material was ranked for effectiveness. Separate rankings were made by each group (Table 2). Based on average ranks, paraffin wax in cetyl alcohol was the most effective material. The sequence of effectiveness was: paraffin wax in cetyl alcohol; >paraffin wax and Acetulan[®] in cetyl alcohol; >paraffin wax and beeswax in cetyl alcohol; >cetyl alcohol; >beeswax in cetyl alcohol; >beeswax and Acetulan[®] in cetyl alcohol; >Acetulan[®] in cetyl alcohol; >untreated control site.

Discussion

Paraffin wax, a mixture of solid hydrocarbons obtained from petroleum having the general formula C_nH_{2n}+2, renders wooden vessels impermeable to water or alcohol, and is used for waterproofing wood, paper, etc. Beeswax, an amorphous substance obtained from bee honeycombs, consisting of layers of straight-chain monohydric alcohols with even-numbered straight carbon chains from C₂₄ to C₃₆ esterified with acids, also having even

numbers of C atoms up to C₃₆, is often used for wax paper, candles, etc. Acetulan[®], a pale yellow, practically odorless liquid, is hydrophobic, practically insoluble in water with no emulsification, and used in cosmetic formulations and shampoos. Cetyl alcohol is a white crystalline material having the general formula C₁₆H₃₄O, obtained from spermaceti (a waxy solid derived from the oil of whales) by saponification. Practically insoluble in water, it is widely used as an emollient, emulsion modifier, and coupling agent.

Paraffin wax in cetyl alcohol was the most effective material; the combination of paraffin wax plus Acetulan[®] in cetyl alcohol and beeswax plus Acetulan[®] in cetyl alcohol produced an excellent protective effect against SLS irritation. In decreasing NH₄OH and urea irritation, only paraffin wax plus beeswax in cetyl alcohol and Acetulan[®] in cetyl alcohol showed a significant effect. Paraffin wax in cetyl alcohol produced protection.

Analyzing the individual protective effect and active ingredients of formulations is an important step for the development of protection products. Advantages of this model include: utilization of human skin in vivo with performance simplicity; and suitability for large-scale screening. This method could be used as a reference system for new barrier cream formulations, and has potential value as a bioengineering technique. The sensitivity of the statistical test was increased among treatments, so small differences could be detected. The data of this study needs further validation with open, non-occlusive studies.

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