

Anti-irritants II: efficacy against cumulative irritation

FLEMMING ANDERSEN^{1,2}, KATHRYN HEDEGAARD², THOMAS KONGSTAD PETERSEN², CARSTEN BINDSLEV-JENSEN^{1,3},
ANN FULLERTON² AND KLAUS EJNER ANDERSEN¹

¹Department of Dermatology and Allergy Center, Odense University Hospital, University of Southern Denmark, DK-5000 Odense C, ²LEO Pharma A/S, Industriparken 55, Ballerup, and ³The Allergy Center, Odense University Hospital, University of Southern Denmark, DK-5000 Odense C, Denmark

So-called anti-irritants (AI) are widely used in cosmetic formulations, with the aim of reducing irritation from substances in the formulation. It may also be claimed that they are 'soothing' and 'healing' ingredients. However, the proof for these claims is circumstantial. The dose-response effect of 4 alleged AI (nifedipine, (-)- α -bisabolol, canola oil and glycerol) was studied on experimentally induced acute irritation in healthy volunteers, and only glycerol showed dose-related response and effects potentially better than no treatment. The acute irritation model only allowed a small window of opportunity in which to demonstrate efficacy. Therefore, the effect of AI was studied in a cumulative irritation model by inducing irritant dermatitis with 10 min daily exposures for 5 + 4 days (no irritation on weekend) to 1% sodium lauryl sulfate on the right and 20% nonanoic acid on the left volar forearm. AI ointments were applied twice daily. Clinical scoring was performed daily, evaporimetry (Trans Epidermal Water Loss), hydration and colourimetry were measured at baseline (D0), in the middle and at the end of treatment. The glycerol ointment was the only treatment statistically better than both 'no treatment' and vehicle.

Key words: anti-irritant; bisabolol; canola oil; cumulative test; glycerol; human; in vivo; nifedipine; nonanoic acid; skin irritation; sodium lauryl sulfate. © Blackwell Munksgaard, 2006.

Accepted for publication 19 September 2005

When Goldemberg defined anti-irritants (AI) 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' (1), he did not intend the term to be used for agents with a 'healing' or 'soothing' effect on already irritated skin. However, so-called AI are being added to cosmetic formulations with the claimed intent to protect and heal sensitive or irritated skin (2), but scientific support of the claim is limited.

In an acute irritation model, 4 alleged AI (nifedipine, (-)- α -bisabolol, canola oil and glycerol) were applied as treatment modalities for experimentally induced skin irritation, and only glycerol demonstrated a dose-related response as well as a treatment effect (3).

The inability of the acute model to demonstrate clear proof of the efficacy of 3 of 4 presumed AI substances prompted us to investigate a cumulative irritation model, which might be more relevant for efficacy studies (4, 5).

The effect of the 4 AI was then studied on experimentally induced cumulative skin irritation by sodium lauryl sulfate (SLS), a corrosive irritant (6) and by nonanoic acid (NON), a non-corrosive irritant (7).

Materials and Methods

Volunteers

36 healthy human volunteers (10 males and 26 females, aged 21–29 years, median age 23 years) were recruited. Subject participated only after receiving verbal and written information about the study and giving their informed consent. The study was approved by the local ethics committee (Den videnskabetiske komite for Vejle og Fyns Amter, VF 20040002) and the Danish Medicines Agency (Lægemiddelstyrelsen, journal no. 2612-2462). Subjects were not included if pregnant or breastfeeding, if in treatment with substances that might influence the effect of the test substances such as immunosuppressants or if

suffering from endocrine or immune system diseases.

Irritants and irritant concentrations

SLS aqueous (>99% purity; Sigma Chemicals Co., St. Louis, MO, USA), a corrosive irritant and NON in n-propanol (ABCR GmbH & Co. KG, Karlsruhe, Germany), a non-corrosive irritant, were used in the study.

In a pilot study, irritant concentrations of SLS 1%, 2% and 3% as well as NON 10%, 20 and 30% were tested on volunteers. Due to severe unwanted effects of the higher concentrations in the pilot as well as in a previous study (8), we chose to use 1% SLS and 20% NON in the ensuing studies.

Treatments

The following agents were chosen for the studies, as they were safe, and studies showing AI effect had been published. The AI have previously been described in detail (3).

Vehicle

Basissalve (DLS), an ointment consisting of 10% polyethylene and 90% paraffin oil, was chosen as vehicle for the AI.

AI

- (1) (-)- α -bisabolol (minimum 95% pure, Cosnaderm, Ladenburg, Germany), 0.5% in basis salve
- (2) Canola Oil (Lipex Canola-U, Karlshamn AB, Karlshamn, Sweden), 40% in basis salve
- (3) Glycerol (Ph.Eur., Karlshamn Tefac AB, Karlshamn, Sweden), 20% in basis salve
- (4) Nifedipine (Ph.Eur., Siegfried CMS AG, Kirchberg, Schweiz), 1.0% in basis salve

Induction of cumulative irritation

A modified open exposure model (8, 9) was used for induction of cumulative irritation in accordance with *Guidelines on sodium lauryl sulphate (SLS) exposure tests* (6). 6 circular areas of 2-cm diameter were outlined on both volar forearms with a felt tip pen. Following baseline measurements on D0, a plastic template with 6 holes corresponding to the above mentioned 6 areas was strapped to both volar forearms. On the right arm, 800 μ l 1% SLS aqueous was pipetted into each opening, and on the left arm, 800 μ l 20% NON in n-propanol was pipetted into each opening. After 10-min exposure, the arms were

rinsed under running lukewarm water, and the arms patted dry with paper towels. This procedure was performed daily Monday through Friday of the 1st week and again Monday through Thursday of the 2nd week.

Application of treatments

An amount of 5 mg of ointment per cm^2 corresponding to a total of approximately 16 mg was applied evenly twice daily (immediately post-application of the irritant and again at least 6 hr later), for 11 consecutive days (D0–D10). The volunteers applied the second daily treatment and all four treatments during the weekend.

Study design and sample size

The study was double blinded with both the subject and the assessor being blind to the treatment modality applied to each site (apart from the untreated site).

With 6 sites and 6 treatment modalities (no treatment, vehicle and 4 AI), treatments could be randomized to sites using a Latin Square Design (10). A cumulative irritation pilot study (12 volunteers) was performed to determine an appropriate sample-size (24, 30 or 36). The standard deviation was found to be larger following cumulative irritation than acute irritation. Given a standard deviation of 7.0, a sample-size of 36 was chosen, ensuring that for a change in Trans Epidermal Water Loss (TEWL) value, there would be 80% power to detect a difference in mean change of 3.5 g/m^2 hr between treatments.

Clinical assessments

Scoring was performed using the European Society of Contact Dermatitis guideline on clinical scoring of subacute/cumulative SLS irritant reactions, elaborate scoring system, with a sum score range of 0–15, with reactions >9 being very strong or caustic (6). Clinical assessment of the test sites was performed once daily prior to application of the irritant and treatment (D0–D4 and D7–D10) and once at End of Treatment (EOT – D11).

Biometric measurements

All measurements were performed prior to treatment and irritant application D0 (baseline), D4 and D11 (EOT).

Colorimetry (a^* -parameter)

The skin colour was assessed by a Minolta colourimeter (CR300, Osaka, Japan). Measurements were

made according to guidelines (11). The a^* -parameter was used to estimate the degree of erythema, as a measure of inflammation. Measurements were performed in triplicate, and the mean a^* value (a.u.) was used.

TEWL

TEWL, a measure of skin barrier integrity, was measured with a DermaLab[®] TEWL probe (Cortex Technology, Hadsund, Denmark). Measurements were done according to guidelines (12, 13).

Cutaneous hydration

Hydration of the horny layer was estimated using a DermaLab[®] Moisture Pin Probe (Cortex Technology), that measures electrical conductance, according to guidelines (14).

Statistics

The biometric assessments on D11 were modelled using Analysis of Covariance (ANCOVA), thus allowing adjustment of the EOT values for the baseline values. The daily clinical score measurements were summarized by constructing the summary measure Area under the Curve (AUC) (15). This summary measure was modelled using Analysis of Variance (ANOVA).

The validity of the assumptions that underlie the ANCOVA and ANOVA models was checked, and where necessary, data were logarithmically transformed or outliers removed.

In both the ANOVA and the ANCOVA models, the factors of interest – treatments and position of test site on the arm – were modelled as systematic factors (i.e. fixed effects). Significance was assumed at the 5% level. Volunteer was included as a random effect in both the ANOVA and ANCOVA models [i.e. a mixed model was used (16)].

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 (17). The analyses have been performed using Proc Mixed, SAS version 8.2. The Kenward-Roger method (18) for adjusting the standard errors and degrees of freedom obtained has been used.

Results

Sample raw data are presented in Fig. 1, Tables 1, 2, 3 and 4.

The results from the ANCOVA and ANOVA models adjusted for both baseline value and position of the test site are summarized in Table 5. The results suggest that glycerol is best, not treating

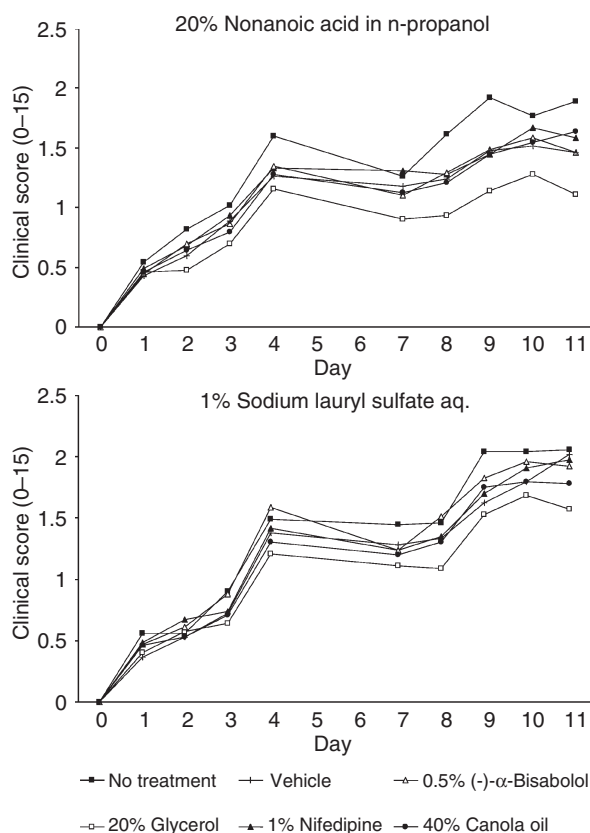


Fig. 1. Development of cutaneous irritation assessed clinically from baseline to end of treatment. Data are given as means.

is worst and there is little difference between the treatments vehicle, canola oil, α -bisabolol and nifedipine. Analysis of the biometric assessments at D4 supported the finding that glycerol ointment was significantly better than no treatment.

Discussion

The reaction patterns to the 2 irritants (Tables 1–4), including the decreasing redness following repeat exposure to NON, due to formation of a plasticized 'film' covering the site obscuring the colourimetric measurements are in accordance with previous

Table 1. Effect of anti-irritants on clinical scores as assessed by area under the curve^a

Formulation	1% Sodium lauryl sulfate, 5 + 4 days	20% Nonanoic acid, 5 + 4 days
Nifedipine cream (N)	13.0 (9.0–16.6)	12.9 (8.0–16.5)
Glycerol cream (G)	10.8 (8.5–14.5)	9.6 (5.4–12.8)
Canola oil cream (C)	12.3 (8.9–15.4)	12.0 (7.0–15.9)
Bisabolol cream (B)	12.0 (8.4–17.9)	12.5 (7.9–16.0)
Vehicle (V)	12.0 (8.3–17.4)	12.0 (7.5–15.5)
No treatment (0)	11.9 (9.6–15.9)	14.0 (10.1–18.6)

^aData are given as median (interquartile range).

Table 2. Baseline and end of treatment colorimetric values^a

	1% Sodium lauryl sulfate 5 + 4 days	20% Nonanoic acid 5 + 4 days
Baseline		
No treatment (0)	7.3 (6.4–8.5)	7.8 (6.8–8.8)
D4		
No treatment (0)	7.2 (6.4–8.5)	7.4 (6.5–7.9)
Vehicle (V)	7.0 (6.2–7.6)	7.1 (6.3–8.0)
20% Glycerol (G)	7.5 (6.0–8.4)	7.0 (6.1–7.5)
End of treatment (D11)		
No treatment (0)	7.2 (5.9–8.8)	6.8 (6.1–8.1)
Vehicle (V)	7.7 (6.5–8.5)	6.8 (5.9–7.6)
20% Glycerol (G)	7.2 (5.9–8.8)	6.4 (5.9–7.5)

^aData are given as median (interquartile range).Table 3. Baseline and end of treatment transepidermal water loss values^a

	1% Sodium lauryl sulfate 5 + 4 days	20% Nonanoic acid 5 + 4 days
Baseline		
No treatment (0)	7.9 (6.5–8.9)	7.6 (6.5–8.5)
D4		
No treatment (0)	11.2 (8.8–12.5)	9.2 (8.1–10.8)
Vehicle (V)	10.8 (8.6–13.5)	8.6 (7.7–10.5)
20% Glycerol (G)	10.5 (8.1–13.1)	7.4 (6.6–9.2)
End of treatment (D11)		
No treatment (0)	14.0 (11.7–16.7)	11.3 (9.9–13.0)
Vehicle (V)	15.2 (12.5–17.4)	10.3 (8.6–12.7)
20% Glycerol (G)	13.7 (10.7–16.1)	8.5 (7.4–11.0)

^aData are given as median (interquartile range).
Units are g/m² h.

studies (8). The flattening of the curve depicting clinical irritation on D5 and D6 followed by an increase from D7 on is due to cessation of irritation in the weekend (Fig. 1). Thus, the model emulates the pattern seen in occupational contact dermatitis, characterized by a reduction in the symptoms on

Table 4. Baseline and end of treatment hydration values^a

	1% Sodium lauryl sulfate 5 + 4 days	20% Nonanoic acid 5 + 4 days
Baseline		
No treatment (0)	53.3 (45–61.7)	53.3 (46.7–65.0)
D4		
No treatment (0)	40.0 (30.0–48.3)	30.0 (23.3–38.3)
Vehicle (V)	43.3 (36.7–58.3)	33.3 (28.3–45.0)
20% Glycerol (G)	63.3 (51.7–80.0)	50.0 (40.0–68.3)
End of treatment (D11)		
No treatment (0)	40.0 (30.0–55.0)	30.0 (26.7–45.0)
Vehicle (V)	41.7 (33.3–58.3)	40.0 (28–53)
20% Glycerol (G)	71.7 (50.0–83.3)	61.7 (46.7–80.0)

^aData are given as median (interquartile range).
Units are μ S.

weekends and holidays, followed by an increase in symptoms when work is resumed.

The finding that 20% NON-induced irritation was better, than 1% SLS-induced irritation, for distinguishing between AI indicates the importance of not relying on 1 model irritant for cutaneous irritation studies.

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 previous studies (3). 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 (8, 19). Even with different vehicles and range of concentrations, (-)- α -bisabolol and canola oil were without any effect.

The set-up described herein was designed as a tool for proof of concept for potential AI. By dimensioning the study to enable detection of a relatively large effect difference (3.5 g/m² hr), only potent AI are identified. By increasing the sample-size, more AI (weaker) may be detected.

Table 5. Statistical ranking of AI by pairwise comparisons with Turkey adjustment in the final models

	Clinical score (AUC)						Colorimetry (a*-parameter)						TEWL (g/m ² h)						Hydration (μ S)					
	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

AI, anti-irritants; AUC, area under the curve; B, (-)- α -bisabolol ointment; C, canola oil ointment; G, glycerol ointment; N, nifedipine ointment; NON, nonanoic acid; O, no treatment; V, vehicle; SLS, sodium lauryl sulfate; TEWL, trans epidermal waterloss.

Underlining of two AI means that they are not statistically 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 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.

In conclusion, we only found support for using 1 of 4 alleged AI as treatment modality for cutaneous irritation, i.e. glycerol. We suggest that prior to making claims about the AI effects of new chemical entities, these should be subjected to testing in human models, in models mimicking contact dermatitis.

Acknowledgements

The authors thank Mette Sonne, LEO Pharma A/S; Ulla Bindslev-Jensen, Centralapoteket, Odense University Hospital and Kirsten Hammond Andersen, Department of Dermatology, Odense University Hospital for their significant contributions to this study.

References

- Goldemberg R L. Use of anti-irritants in cosmetic formulating. *J Soc Cosmet Chemists* 1965; 16: 317–340.
- Goldemberg R L. Anti-Irritants. *J Soc Cosmet Chemists* 1979; 30: 415–427.
- Andersen F, Hedegaard K, Petersen T K, Fullerton A, Bindslev-Jensen C, Andersen K E. Anti-Irritants I: dose-response in acute irritation. *Contact Dermatitis* 2006; 55: 148–154.
- Malten K E. Thoughts on irritant contact dermatitis. *Contact Dermatitis* 1981; 7: 238–247.
- Tupker R A, Bunte E E, Fidler V, Wiechers J W, Coenraads P J. Irritancy ranking of anionic detergents using one-time occlusive, repeated occlusive and repeated open tests. *Contact Dermatitis* 1999; 40: 316–322.
- Tupker R A, Willis C, Berardesca E, Lee C H, Fartasch M, Agner T, Serup J. Guidelines on sodium lauryl sulfate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1997; 37: 53–69.
- Wahlberg J E, Lindberg M. Nonanoic acid – an experimental irritant. *Contact Dermatitis* 2003; 49: 117–123.
- Andersen F, Hedegaard K, Petersen T K, Fullerton A, Bindslev-Jensen C, Andersen K E. The hairless guinea pig as a model for treatment of cumulative irritation in humans. *Skin Res Technol* 2006; 12: 60–67.
- Tupker R A, Vermeulen K, Fidler V, Coenraads P J. Irritancy testing of sodium laurate and other anionic surfactants using an open exposure model. *Skin Res Technol* 1997; 3: 133–136.
- Cochran W, Cox G. Completely Randomised, Randomised Block and Latin Square Designs. In: *Experimental Designs*, New York, Wiley Publications in Statistics Wiley, 1957: pp. 117–127.
- Fullerton A, Fischer T, Lahti A, Wilhelm K P, Takiwaki H, Serup J. Guidelines for measurement of skin colour and erythema. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1996; 35: 1–10.
- Pinnagoda J, Tupker R A, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1990; 22: 164–178.
- Rogiers V, EEMCO Group. EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. *Skin Pharmacol Appl Skin Physiol* 2001; 14: 117–128.
- Berardesca E. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. *Skin Res Technol* 1997; 3: 126–132.
- Matthews J N S, Altman D G, Campbell M J, Royston P. Analysis of serial measurements in medical research. *BMJ* 1990; 300: 230–235.
- Brown H, Prescott R. *Normal Mixed Models. Applied Mixed Models in Medicine: Statistics in Practice*. Chichester, Wiley, 1999: pp. 36–40.
- Hochberg Y, Tamhane A C. *Single-Step Procedures for Pairwise and More General Comparisons Among All Treatments. Multiple Comparison Procedures*, Wiley Series in Probability and Mathematical Statistics. New York, Wiley, 1987: pp. 91–93.
- Kenward M G, Roger J H. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 1997; 53: 983–997.
- Andersen F, Hedegaard K, Petersen T K, Fullerton A, Bindslev-Jensen C, Andersen K E. The hairless guinea pig as a model for treatment of acute irritation in humans. *Skin Res Technol* 2006; 12: 183–189.

Address:

Dr Flemming Andersen
Department of Dermatology
Odense University Hospital
Sdr. Boulevard 29
DK-5000 Odense C
Denmark
Tel: +45 6541 3239
Fax: +45 6612 3819
e-mail: fmland@stofanet.dk