Evaluation of the effect of Dardia® Lipo Line on skin inflammation induced by surfactants using the repeated open-application test

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Keywords
Dardia, dry skin, hydration, irritation, sensitive

Abstract

Background Medical skin care products are topical preparations with mainly moisturizing properties. A new line of medical skin products with an excellent tolerability profile and improved hydration for dry skin has been developed, but beneficial effects have not yet been investigated on damaged skin.

Aim To investigate if these products maintain barrier function and hydration status, improve subjective symptoms due to irritant contact dermatitis and to prove their tolerability on damaged skin.

Design and methods Single-centre, blinded, randomized, controlled study in 20 healthy Caucasian women. 5% sodium lauryl sulphate solution was used to induce skin irritation. Two sites on the inside surface of both forearms of each subject were treated daily for 5 days (irritation period). Lipo Cream, Lipo Milk (water-in-oil emulsions) and Lipo Ointment (water-free formulation) were applied twice daily to three of the four test sites on days 1–5. The fourth site was used as a control. Visual readings, subjective symptom assessments, transepidermal water loss (TEWL) and colorimetric measurements, corneometry and skin microrelief macrophotographies were done on days 1–6.

Results On day 6, TEWL was increased vs baseline on all sites; however, TEWL with Lipo Cream or Lipo Ointment was significantly lower than control. At day 6, skin capacitance was 94%, 100% and 85% of baseline value for the cream, milk and ointment, respectively, versus 72% for control. All test products were well tolerated.

Conclusions Lipo Line products showed both protective properties against epidermal dysfunction and significant hydrating effect.

Introduction
Medical skin care products are topical preparations with mainly moisturizing properties in addition to other beneficial cosmetic features. Although they do not contain pharmacologically active ingredients, they are very useful in dermatological practice: they are used either in the treatment of dermatological diseases as an adjunct to treatment and in the post-therapeutic phase (e.g. atopic dermatitis, psoriasis, ichthyosis, etc.), or they provide some protection to healthy skin exposed to environmental irritants. They provide more safety than standard cosmetics in terms of irritancy and allergenic risk and are thereby recommended by dermatologists for daily skin care.

A new medical skin care line (Lipo Line) consisting of three skin care products has been developed. Initial studies have indicated that the Lipo Line range provides an excellent tolerability profile and improved hydration on dry skin (4-week use tests; data on file, Intendis GmbH). However, beneficial effects of Lipo Line products have not yet been shown on damaged skin.

The repeated open-application test (ROAT) with sodium lauryl sulphate (SLS) offers a good simulation of conditions in daily practice as it mimics repetitive and cumulative exposure to environmental irritants and allows the effect of moisturisers on the surfactant-induced skin irritation and skin repair to be studied. This chronic model seems to represent the clinical situation of irritant contact dermatitis.
with pronounced skin dryness more closely than the acute irritation model.¹

This study was designed to investigate if the Lipo Line products would be able to maintain the skin barrier function and skin hydration status, improve subjective symptoms due to irritant contact dermatitis and prove their tolerability on damaged skin, allowing their recommendation for environmentally sensitive/reactive skin.

**Methods**

The study was a single-centre, randomised (application sites), controlled (untreated site), investigator-masked study, with intra-individual comparisons.

**Patient selection**

Volunteers underwent a 2-week screening period (between day 14 and day 1) to assess the eligibility of the subject. During the first screening visit, the medical/surgical/drug history of the subject was recorded and a physical examination (including vital signs) was done.

Female subjects in good health, with normal findings in their medical history and physical examination, 18 to 45 years of age, phototype II to III and of Caucasian origin were considered for inclusion in this study. All subjects had a history of skin damage after contact with environmental agents (reactive skin).

Pregnant women, nursing mothers, women planning a pregnancy during the course of the trial or women not taking adequate contraceptive measures were excluded from this trial. Subjects were also excluded if they had any surgical or medical condition that, in the judgement of the investigator, might interfere with the study, or were receiving local or systemic corticotherapy or any type of anti-inflammatory treatment which might interfere with the results of the study (investigator’s judgement). Known allergies to any of the ingredients of the Lipo Line products, any cutaneous disorder at experimental sites and cutaneous diseases such as psoriasis or active atopic dermatitis (atopic diathesis was not an exclusion criteria), even at sites other than the experimental ones, were criteria for exclusion.

The study was done in accordance with the relevant guidelines of the Declaration of Helsinki. All subjects provided informed written consent.

**Patient instructions**

Each subject was informed of specific requirements or instructions to follow while participating in the study. They were asked to refrain from wetting the test sites: bathing, swimming and vigorous exercise that might result in excessive sweating were not authorized during the study. All subjects were asked to strictly avoid exposure to the sun or artificial ultraviolet light, particularly on the forearms.

For test product applications at home, the subject was given disposable syringes containing the test products with the instructions for applications (including a drawing representing the forearms with the numbered test zones) at the end of each visit day. The syringes were filled each morning by the product manager.

**Protocol**

The first 5 days of the study were termed the irritation period (days 1–5). Skin irritation was induced on the inner surface of the two forearms of healthy volunteers daily for five consecutive days. From day 1 to day 5 in the morning, 100 µL of SLS 5% (in distilled water) was applied on 20-mm diameter filter paper discs on two symmetrical skin sites of the inner side of each forearm (two per forearm, four sites in total per subject) per tested product and kept occluded under Large Finn Chambers® for 30 min. After the 30 min of SLS application, the skin was gently wiped and dried. Then, after 30 min, Lipo Cream, Lipo Milk and Lipo Ointment were applied without occlusion according to a randomization scheme on three sites. The products were applied directly onto the skin and gently massaged to ensure penetration. The fourth site was left untreated as a control. The tested sites were left open without any guard or dressing, and the test products were applied once again in the evening at home by the subjects.

In order to monitor skin reactions, visual readings, subjective symptom assessments, transepidermal water loss (TEWL) and colorimetric measurements, corneometry and skin microrelief macrophotographies were done daily, before application of the SLS on the designated sites.

At the final visit (day 6), all assessments were done approximately 24 h after the last SLS application.

**Materials**

The 5% SLS solution was prepared at the investigational site using sterile distilled water and SLS (Sigma).

The three test substances are Lipo Cream, Lipo Milk and Lipo Ointment. Their respective vehicle constituents are shown in Table 1.

**Randomization**

The products were applied topically on the skin (i.e. on selected test zones on both forearms and according to a randomization list). All the subjects received all three investigational products described above.
Upon enrolment, each subject was assigned a unique number (subject identifier) corresponding to the chronological entrance into the study. The subject’s number corresponded to the allocation of three test formulations to three of the four test zones (one test zone remained untreated) according to a randomization list. The test zones were numbered as indicated in fig. 1. The test zones were located at least at 4 cm from the elbow and wrist folds. A 5-cm minimum interval was respected between both test zones on the same forearm.

The test zones were located at least at 4 cm from the elbow and wrist folds. A 5-cm minimum interval was respected between both test zones on the same forearm. The randomization list was prepared by a biostatistician independent of the study using the SYSTAT version 11.0 statistical software (SYSTAT Inc., 1800 Sherman Ave, IL). All trial personnel, with the exception of the product manager and the biostatistician who prepared the randomization list, were blinded until the database was locked. The randomization list was kept strictly confidential, accessible only to authorized persons, until the time of unblinding. Only once the trial was completed, the data file verified, the protocol violations determined and the database locked were the drug codes broken and made available for data analysis.

**Measurements**

The primary efficacy variables were the TEWL values (g/m²/h), the composite total visual score and the composite subjective sum score (see Tables 2 and 3). The TEWL measurements were done using Tewameter 300 (Courage and Kazakha, Köln, Germany). The measure

<table>
<thead>
<tr>
<th>Formulation name</th>
<th>Vehicle ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipo Cream</td>
<td>Aqua, Caprylic/Capric Triglyceride, Polyglyceryl-3 Diisostearate, Glycerin, Dicaprylyl Ether, Cetearyl Ethylhexanoate, Petrolatum, Cera Alba, Sodium Lactate, Polyglyceryl-2 Dipolyhydroxystearate, Magnesium Sulphate, Lactic Acid, Ethylhexylglycerin</td>
</tr>
<tr>
<td>Lipo Milk</td>
<td>Aqua, Caprylic/Capric Triglyceride, Cetearyl Ethylhexanoate, Urea, Polyglyceryl-2 Dipolyhydroxystearate, Sodium Lactate, Glycerin, Dicaprylyl Ether, Polyglyceryl-3 Diisostearate, Lactic Acid, Magnesium Sulphate, Ethylhexylglycerin</td>
</tr>
<tr>
<td>Lipo Ointment</td>
<td>Petrolatum, Paraffinum Liquidum, Microcrystalline Wax, Oleyl Erucate, Urea, Corn (Zea Mays) Starch</td>
</tr>
</tbody>
</table>

**Table 2** Visual scores

These scores were added together to produce a composite total visual score

<table>
<thead>
<tr>
<th>Erythema</th>
<th>0. No erythema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Slight redness, spotty or diffuse</td>
</tr>
<tr>
<td></td>
<td>2. Moderate, uniform redness</td>
</tr>
<tr>
<td></td>
<td>3. Intense redness</td>
</tr>
<tr>
<td></td>
<td>4. Fiery red with oedema</td>
</tr>
<tr>
<td>Scaling</td>
<td>0. No scaling</td>
</tr>
<tr>
<td></td>
<td>1. Fine scaling</td>
</tr>
<tr>
<td></td>
<td>2. Moderate scaling</td>
</tr>
<tr>
<td></td>
<td>3. Severe scaling with large flakes</td>
</tr>
<tr>
<td>Fissures</td>
<td>0. No crack/fissure</td>
</tr>
<tr>
<td></td>
<td>1. Fine cracks</td>
</tr>
<tr>
<td></td>
<td>2. Single or multiple broader fissures</td>
</tr>
<tr>
<td></td>
<td>3. Wide cracks with haemorrhage or exudation</td>
</tr>
</tbody>
</table>

**Table 3** Subjective symptoms (dryness, pruritus and burning sensation)

These scores were assessed on a 5-point scale from 0 to 4 and were added together to produce a composite subjective score

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.</td>
<td>No symptoms</td>
</tr>
<tr>
<td>1.</td>
<td>Minimal symptoms</td>
</tr>
<tr>
<td>2.</td>
<td>Mild symptoms</td>
</tr>
<tr>
<td>3.</td>
<td>Moderate symptoms</td>
</tr>
<tr>
<td>4.</td>
<td>Severe symptoms</td>
</tr>
</tbody>
</table>
of the cutaneous evaporation rate was expressed in g/m²/h and was taken after the stabilization of the value (about 1 min after the probe was applied on the skin). Measurements were done in a room where temperature and relative humidity were stable. Temperature (between 21 °C and 24 °C) and relative humidity (between 40% and 60%) were recorded for each measurement.

Secondary efficacy variables included colorimetric parameters (L*, a*, b*), corneometry (hydration) and skin microrelief macrophotographies (dermatoscopy). The skin colour measurements were done using a Minolta Chroma meter CR 300 (Minolta, Tokyo, Japan). For corneometry, the CORNEOMETER CM 820® (Courage & Khazaka) was used. This instrument allows assessment of the hydration state of the first horny layers using electrical capacitance measurements. Three measurements were taken on each site, respectively, and were averaged for further evaluation. The Dermatoscope FOTOFINDER® (DERMA, Bad Birnbach, Germany) equipped with a digital connection to a PC on which dedicated image analysis software was installed and was used for the dermatoscopy; the magnification was set at ×20.

Local and systemic adverse events were recorded as reported by the patient and observed by the investigator. The observation of adverse events and recording of concomitant medication were documented throughout the study. Adverse events were classified as mild (awareness of sign or symptom, but easily tolerated), moderate (discomfort, enough to cause interference with usual activity) or severe (incapacitating with inability to work or perform usual activity).

Results

Twenty healthy Caucasian females with a history of skin damage with environmental agents, aged 33 ± 9 years (range 18–45 years), were enrolled and completed the study. All the subjects were fully informed and signed a consent form before inclusion. The study was carried out in October and November 2006.

The per-protocol analysis set consisted of all 20 randomised subjects.

Table 4 EWL mean values ± SD (g/m²/h) at baseline and on the last study day (day 6). Calculated AUC means ± SD

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Lipo Cream</th>
<th>Lipo Milk</th>
<th>Lipo Ointment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8.39 ± 2.45</td>
<td>8.48 ± 2.12</td>
<td>8.35 ± 1.92</td>
<td>8.20 ± 2.16</td>
</tr>
<tr>
<td>Day 6</td>
<td>26.24 ± 15.66</td>
<td>21.16 ± 15.12*</td>
<td>25.71 ± 17.22</td>
<td>21.30 ± 12.95*</td>
</tr>
<tr>
<td>AUCday 1–day 6</td>
<td>72.26 ± 34.28</td>
<td>69.37 ± 37.95</td>
<td>76.45 ± 40.54</td>
<td>67.78 ± 34.26</td>
</tr>
</tbody>
</table>

*Pairwise comparison with untreated but SLS-exposed control, P < 0.001.
The four test sites had equivalent baseline capacitance values \((P = 0.45)\). Baseline values were lower than the normal values indicating physiological dry skin.

The effect of the repeated SLS contact on skin hydration was highlighted by the corneometry measurements (capacitance). After one application (day 2) and after two applications (day 3), the mean capacitance values on the untreated but SLS-exposed site decreased and were, respectively, equal to 81% and 67% of the baseline value (fig. 3). Twenty-four hours after the first SLS application, the capacitance measurements on the Lipo Milk and the Lipo Cream treated sites were increased by 22% and 10%, respectively, compared with baseline values, after two product applications. Despite repeated SLS applications, the three Lipo Line products significantly preserved the normal moisture content of the skin. This effect was statistically significant for all post-baseline visits and for the calculated AUC \((P < 0.001)\). At day 6, the mean capacitances obtained with Lipo Cream, Lipo Milk and Lipo Ointment were, respectively, equal to 94%, 100% and 85% of the baseline value compared with 72% for the untreated but SLS-exposed site. Lipo Milk produced the most pronounced protective effect against the SLS drying effect.

The efficiency of Lipo Cream, Lipo Ointment and Lipo Milk against chemically induced skin dryness was proven.

**Colorimetric measurements**

The colorimetric parameter \(a^*\), which is an expression of erythema, measured on the four sites differed at baseline \((P = 0.002)\). The post-baseline values were adjusted to baseline before analysis.

During the study, no significant difference was shown between the four test sites. However, the results for this parameter showed the same numerical ranking as for TEWL. SLS-induced erythema was more pronounced on the control site than on the treated sites. The most numerically active product was the Lipo Cream with an increase of the \(a^*\) value (Delta \(a^*\)) of 2.36 compared with 3.76 on the untreated site at the end of the study.

**Clinical assessments and subjective symptoms**

A composite total clinical score (TCS) was determined by adding the three clinical scores: erythema, scaling and fissures. A composite total subjective score (TSS) was obtained by adding the three subjective symptom scores: dryness, pruritus and burning sensation. The TCS and the TSS could be 0 to 9. The lowest TCS (day 6) was obtained with the Lipo Cream (1.0 ± 1.6) compared with the untreated control (1.7 ± 1.3). This is in line with the results of the erythema objective measurements (Delta \(a^*\)). However, under the study conditions, the very low number of positive scores did not allow any statistical comparison. The TCS and TSS data are summarized in Table 6.

**Table 5** Capacitance mean values (arbitrary unit, AU) at baseline and on the last study day (day 6). Calculated AUC means ± SD

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Lipo Cream</th>
<th>Lipo Milk</th>
<th>Lipo Ointment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>31.22 ± 7.28</td>
<td>31.13 ± 6.99</td>
<td>30.02 ± 8.09</td>
<td>31.02 ± 6.72</td>
</tr>
<tr>
<td>Day 2</td>
<td>25.43 ± 6.75</td>
<td>34.20 ± 7.21**</td>
<td>36.70 ± 8.47**</td>
<td>30.82 ± 7.78**</td>
</tr>
<tr>
<td>Day 6</td>
<td>22.55 ± 8.88</td>
<td>29.13 ± 9.66**</td>
<td>30.05 ± 12.57**</td>
<td>26.42 ± 7.21*</td>
</tr>
<tr>
<td>AUC_Day 1-Day 6</td>
<td>116.78 ± 28.21</td>
<td>146.50 ± 26.16**</td>
<td>158.83 ± 33.23**</td>
<td>140.28 ± 28.00**</td>
</tr>
</tbody>
</table>

*Pairwise comparison with untreated site, \(P < 0.05\); **Pairwise comparison with untreated site, \(P < 0.001\).

**Table 6** Clinical assessments and subjective symptoms at day 6

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Lipo Cream</th>
<th>Lipo Milk</th>
<th>Lipo Ointment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCS</td>
<td>1.7 ± 1.3</td>
<td>1.0 ± 1.6</td>
<td>1.5 ± 1.6</td>
<td>1.1 ± 1.1</td>
</tr>
<tr>
<td>TSS</td>
<td>1.0 ± 1.1</td>
<td>1.2 ± 1.9</td>
<td>1.2 ± 1.3</td>
<td>0.9 ± 1.5</td>
</tr>
</tbody>
</table>

TCS: Sum of erythema, scaling and fissures; TSS: Sum of dryness, pruritus and burning sensation.
Safety

All the test products were well tolerated. Thirteen adverse events were reported by the 20 enrolled subjects. No adverse event was related to the products.

Discussion

The skin is an important organ of defence to the environment. However, it is vulnerable to physical, chemical and biological agents. Cutaneous hazards of chemical source are largely present in consumer products. Repeated skin exposure to irritants is very frequent in daily life. Kligman showed that some people exposed to occupational irritant-induced chronic dermatitis have vulnerable skin (hyper-reactors). Surfactants cause skin irritation in a great number of people who are exposed to these agents frequently. SLS has been used extensively as a model irritant in the field of skin irritation testing. This irritant allows for precise testing and highly sensitive measurement of response by measurement of TEWL. Increase in TEWL is an early sensitive sign of skin irritation. Extensive literature shows that TEWL measurement is a highly sensitive and precise measurement of SLS irritant effect on the skin. When irritant contact dermatitis is caused by surfactants (such as SLS), changes in TEWL are detected long before any clinical signs as dryness, scaling or erythema. However, TEWL is not a clinical sign or symptom and must be compared with clinical observations.

The course of changes in TEWL, in capacitance measurement (skin hydration) and colorimetric measurements (skin redness), induced by a single SLS application, has been used extensively as a model irritant in the field of skin irritation testing. This irritant allows for precise testing and highly sensitive measurement of response by measurement of TEWL. Increase in TEWL is an early sensitive sign of skin irritation. Extensive literature shows that TEWL measurement is a highly sensitive and precise measurement of SLS irritant effect on the skin. When irritant contact dermatitis is caused by surfactants (such as SLS), changes in TEWL are detected long before any clinical signs as dryness, scaling or erythema. However, TEWL is not a clinical sign or symptom and must be compared with clinical observations.

The study was intended to assess the protective effects of three products from Lipo Line against the skin damage induced by surfactant on vulnerable skin. The short contact time (30 min) and the rather high concentration of SLS (5%) had been shown to be helpful in detecting changes in the skin barrier properties. Similar non-invasive methods to those previously described in the literature were used to detect SLS and product effects on the skin of the inner forearm. TEWL was the primary endpoint, reflecting the skin barrier function.

The baseline TEWL values of our test population were slightly greater than normal values, whereas baseline impedance values were lower (dry skin). These findings are in line with published data. In one study intended to examine if the physiological properties of vulnerable skin differ from those of normal skin, the effects of baseline electrical impedance and TEWL on the reactivity to SLS were studied in patients with a history of contact dermatitis and compared with results in a healthy control group. SLS was applied to the test sites of normal-looking skin on the middle of the inner forearms. Patients with a history of contact dermatitis showed higher TEWL and lower impedance magnitude index on day 3 after exposure to SLS (2% under occlusion) than the controls. Patients prone to irritant contact dermatitis could have a compromised skin barrier with increased TEWL and a dry skin expressed by low capacitance.

In this study, the application of the test products resulted in significantly lower increase of the TEWL (Lipo Cream and Lipo Ointment) and also a significantly higher skin hydration (capacitance) in comparison with the untreated but SLS exposed control field.

The test products contained low molecular weight humectants such as urea (Lipo Ointment), glycerine and lactic acid (Lipo Cream and Lipo Milk). It is assumed that these substances are absorbed in the stratum corneum, and there attract water and increase the degree of hydration. Urea-containing moisturisers influence the skin reactivity to topically applied surfactant (SLS). In a double-blind vehicle-controlled comparison, urea was found to decrease the skin susceptibility to SLS and to increase the skin capacitance after only three applications. This was considered as possibly clinically relevant in reducing contact dermatitis from irritant stimuli.

A panel of healthy volunteers were exposed twice daily for 4 days to alpha-hydroxy, citric, malic and lactic acids, either alone or in tandem application with 0.5% SLS in a repetitive irritation test. Combined exposure to one of the fruit acids and SLS caused marked barrier disturbance, but the latter irritant effect was smaller than that obtained by combined exposure to SLS and water.

Clinical appearance with erythema, scaling and fissuring is seen during repeated application of SLS. No significant clinical irritant was detected in this study. However, the SLS exposition period was relatively time limited. Moreover, chronic irritant contact dermatitis is frequently a phenomenon sensed by the patient but not always physically evident to the observer.

As observed in this study, subjects with skin vulnerable to chemicals probably suffer from a physiologically impaired barrier function and dry skin. It has also been
demonstrated than the barrier function of uninvolved skin in patients with active atopic dermatitis (AD) was compromised as compared with control skin. The TEWL was higher in patients with AD compared with that of the control group (mean ± SD: 8.4 ± 4.3 and 6.3 ± 2.0 g/m²/h, respectively). The diffusivity of SLS through uninvolved AD skin was higher compared with normal skin, whereas the partition coefficient between SC and water was lower. Use of anti-irritant emollients should be recommended also for these patients.

To summarize, the study showed the beneficial effects of Lipo Line products on damaged skin. The three products showed protective properties against epidermal dysfunction and significant hydrating effect. Daily use of Dardia® Lipo Line products should be beneficial to people with chemically vulnerable or delicate skin.

Acknowledgements
This study was funded by Intendis.

Conflict of Interests
JP Ortonne has acted as a paid speaker to Intendis.

References
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