Placebo-Controlled, Double-Blind, Randomized, Prospective Study of a Glycerol-Based Emollient on Eczematous Skin in Atopic Dermatitis: Biophysical and Clinical Evaluation

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Key Words
Atopic dermatitis • Emollient • Glycerol • Moisturizer • Transepidermal water loss

Abstract

Background/Aims: Atopic dermatitis (AD) is a frequent, chronic inflammatory disease influenced by local, immunological, genetic and environmental factors. Important symptoms of AD are dry skin, intense pruritus and impaired epidermal barrier function. The therapeutic management of AD is difficult and needs individualized concepts. Moisturizing creams and emollients are useful and important treatment adjuncts for the daily skin care of patients with dry and inflamed skin, e.g. AD. Glycerol is known to increase stratum corneum (SC) hydration, improve epidermal barrier function and decrease clinical signs of inflammation. However, no controlled study on the efficacy of glycerol on barrier function and SC hydration in AD has been published. In the present study, a topical 20% glycerol preparation was compared with its vehicle in patients with AD. The aim of the present study was to evaluate the effect of a single emollient ingredient in AD within the full frame of a phase III drug study.

Methods: 24 patients with AD were treated for 4 weeks twice daily with a glycerol-based emollient in a randomized, double-blind study. Transepidermal water loss, skin capacitance, erythema and skin surface pH were assessed with biophysical, non-invasive instruments. The SCORAD and a local severity score were evaluated. After a wash-out period of 2 weeks, these parameters were assessed in order to quantify the sustained effect of this treatment. Results: SC hydration was significantly improved, and epidermal barrier function was restored under treatment with glycerol-containing cream compared to the glycerol-free placebo. No significant differences were detectable for erythema values, SCORAD and local severity between the glycerol-containing cream and placebo. However, an improvement over time was detectable in the assessed parameters in both groups indicating the importance of emollient treatment in AD.

Conclusions: Glycerol-based emollients have a positive influence on the skin of patients with AD. They enhance the SC hydration. Furthermore, it was possible to evaluate skin care products with a protocol design for efficacy studies of fully registered drugs in a placebo-controlled study.

Introduction

Atopic dermatitis (AD) is a frequent and chronic inflammatory disease influenced by local, immunological, genetic and environmental factors [1, 2]. Important symp-
toms of AD are dry skin, intense pruritus and impaired epidermal barrier function. Recent studies could show the importance of a mutation of the profilaggrin-encoding genes [3, 4]. Chronic itching and scratching induces skin thickening and lichenification. The disease continues in phases depending on the age of the patient.

Therapy of AD is difficult and needs individual concepts. The continuous skin care in non-acute phases and as an adjuvant to classical treatments is very important since the impaired epidermal barrier and stratum corneum (SC) desiccation play a central role in disease activity [5–7].

Emollients are regularly used in the treatment and skin care of atopic patients. A frequently used moisturizing compound is glycerol. It improves the hydration of the SC due to its high degree of hydroxyl groups, which bind and retain water. Furthermore, an acceleration of barrier recovery of an altered epidermal barrier function has been reported [8]. Glycerol has a keratolytic effect by desmosome degradation [9]. It also influences the protective function of the skin against irritation and penetration of substances through the SC, plasticizes the SC, reduces tissue scattering, stabilizes skin collagen and accelerates healing processes [10].

Glycerol-based emollients are generally considered skin care or cosmetic products. Thus, normally no specific demands on the design of the study like registration by competent authorities except approval by the local ethical committees are required. The present study was designed with a phase III drug registration protocol. The ‘Note for Guidance of Good Clinical Practice for Trials on Medicinal Products in the European Community’ was applied and might be directed not only at medicinal but also dietary products, therapeutic measures and cosmetics. In consideration of the increasing requirements for safety and efficacy of medical products and the prohibition of animal testing, we decided to evaluate the glycerol-based emollient with a protocol designed for efficacy studies of drugs as a placebo-controlled, double-blind, randomized phase III study.

The purpose of the described study was to prove that a glycerol-containing emollient for topical therapy for patients with mild AD on the forearm has a significantly better outcome than the glycerol-free vehicle. The evaluation end-points SC hydration, epidermal barrier function, surface pH, erythema index and clinical severity scores were selected in order to obtain a complete picture of normalization of skin physiology.

Material and Methods

General Description of the Study

The present study was a placebo-controlled, double-blind, randomized phase III study carried out between November 15, 2004, and March 23, 2006. No patients were enrolled between May 1 and August 31, 2005, in order to limit the climatic influence on the study outcome.

Study Population

Twenty-four patients with AD (selected by assessing the Erlangen atopy score with a score >10) and mild to moderate local severity of eczema of both forearms participated in the study (8 female and 16 male patients) [11]. The patients had no other significant concurrent illness and no known allergy to ingredients of the test creams. The mean age of the participants was 23 years (minimum 15, maximum 49 years). The sociographic data of both groups were comparable. All 24 volunteers completed the study. No topical or systemic treatment was administered 2 weeks prior to inclusion of the patients during the study and the wash-out phase. The eczema on both forearms was of comparable severity (see baseline values of local severity score below).

Composition of the Emollients

The glycerol cream contained 20% glycerol (200 mg/g) and the following ingredients according to the International Nomenclature of Cosmetic Ingredients (INCI): aqua, cetearyl alcohol, isopropyl myristate, paraffinum liquidium, PEG-40 hydrogenated castor oil, glyceryl behenate, glyceryl dibehenate, tribehenin, citric acid, sodium citrate, methylparaben, propylparaben. The composition of the placebo was identical without glycerol.

Application of Test Products

The application of test cream and placebo was performed, after extensive explanation at the clinic, by the patients at their homes. According to a randomization list, the right or left forearm was selected for application of the test product. A control area on the untreated contralateral arm was assessed. Thus, each patient served as his own control.

The glycerol-containing cream or the placebo was applied twice per day to the test area for 4 weeks. The amount of cream was determined with fingertip units (FTU) [12]. 1 FTU was used for the insides of the forearm; 2–3 FTU were used when the complete forearm was affected. This amount corresponds roughly to the usual 2 mg/cm² used in clinical trials. A wash-out period of 2 weeks followed after the last application in order to assess the sustained effect of the glycerol formulation. The returned tubes were weighed after the study. The amount of remaining emollient in the tubes did not show a significant difference (p = 0.5068).

Biophysical Assessment of Skin Physiology

The skin physiology parameters were assessed 12 h after the last application of the test products. The same evaluator (trained dermatologist) performed all instrumental evaluations in the same room. The measurements were carried out under standardized ambient conditions (air conditioning, room temperature between 20 and 22°C and relative humidity between 30 and 40%, adaption period with a minimum of 15 min). Barrier function in terms of transepidermal water loss (TEWL) was measured using...
the Tewameter TM 300 (Courage & Khazaka, Cologne, Germany) according to the published guidelines [13, 14]. Two recordings were made and expressed as a mean value. The SC hydration was determined by a capacitance-based corneometer (Corneometer CM 825, Courage & Khazaka) according to the published guidelines [15]. Three recordings from each test area were performed, and the mean value was calculated. The erythema was measured using the Mexameter MX 16 (Courage & Khazaka) [16]. Three recordings were made and expressed as mean value. The pH value was determined with the skin pH-meter PH 900 (Courage & Khazaka) [17]. Measurements were expressed as mean value of 3 readings. The SC integrity and cohesion were determined by sequential tape stripping with D-Squames (20 times; standard 2.1-cm diameter D-Squames, Cuderm, Dallas, Tex., USA). The SCORAD [18] and a local severity score on the volar forearm were determined. For estimation of local severity, a score was calculated based on the severity scoring of AD (SCORAD) system. The symptoms of erythema, oedema/papule, weep/crust, excoriation, lichenification and local pruritus were detected on both volar forearms. Each symptom was valued as 0 = not existing, 1 = mild, 2 = moderate or 3 = severe. The local severity value is calculated as the amount of the single values with a maximum of 18 points.

**Statistics**

The size of the study groups (2 × 12 patients) was determined prior to the beginning of the study by power calculation (Statmate 2.0, San Diego, Calif., USA).

The handling of the results was done with Microsoft Excel 2000. The statistical analysis was carried out with Prism 3.02 for Windows (Graphpad, San Diego, Calif., USA). Differences between the parameters were checked for significance setting p values at ≤ 0.05 with analysis of variance (ANOVA) followed by a post-hoc Bonferroni-adjusted pair-wise comparison.

**Registration Procedure**

Local Ethics Committee

The study was presented and approved by the local ethics committee of the Friedrich Schiller University Jena with approval No. 1436-11/04.

Competent Authorities

The competent authorities for this study were the German FDA (Bundesinstitut für Arzneimittel und Medizinprodukte – BfArM) and the Thuringian authority for food and product safety (Landesamt für Lebensmittelsicherheit und Verbraucherschutz). The study was declared to these competent authorities. The intention of the study was a full phase III drug efficacy study, although the active ingredient is registered as cosmetic ingredient (INCI No.: E422). Therefore, a EudraCT number was requested (2004-004443-22). Thereafter the applicant forms were created in an online dialog with the EudraCT database and submitted. The BfArM approved the study, and the investigation was carried out. After completing the study, the competent authorities were again informed about the successful end of the study.

**Results**

**Skin Physiological Parameters**

Improvement of SC Hydration

Already after 1 week of treatment with the glycerol cream, a statistically significant difference between the two creams was detectable. The application of the glycerol cream led to an improvement of the SC hydration (increased water content – higher capacitance values) in comparison with the application of the cream without glycerol. In the wash-out period after the end of application, the corneometry values of patients previously treated with the glycerol-containing cream remained higher than in the placebo group (fig. 1, table 1).

Stabilization of Barrier Function in the Glycerol-Treated Area

From day 1 on, the glycerol-containing cream led to a discrete, but not significant improvement of the barrier function, measured as lower TEWL values on the forearm of patients treated with glycerol than on patients treated with placebo. TEWL values of the patients previously treated with the glycerol-containing cream were lower than those of the patients of the comparison group.

**Table 1. Statistical results for corneometry**

<table>
<thead>
<tr>
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<th>p values</th>
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<tr>
<td>ANOVA over time</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>&lt;0.0001</td>
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<td>Control to glycerol</td>
<td>0.7074</td>
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<tr>
<td>Placebo</td>
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<tr>
<td>Control to placebo</td>
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<td>ANOVA over groups</td>
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<td>T0</td>
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<tr>
<td>T1</td>
<td>0.0001</td>
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<tr>
<td>T2</td>
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<tr>
<td>T3</td>
<td>0.0001</td>
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<tr>
<td>T4</td>
<td>0.0003</td>
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<tr>
<td>T5</td>
<td>0.8009</td>
</tr>
<tr>
<td>T6</td>
<td>0.5858</td>
</tr>
<tr>
<td>Unpaired t test glycerol versus placebo</td>
<td></td>
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<tr>
<td>T1</td>
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<td>T2</td>
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<tr>
<td>T3</td>
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</tr>
<tr>
<td>T4</td>
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</tr>
<tr>
<td>T5</td>
<td>0.2737</td>
</tr>
<tr>
<td>T6</td>
<td>0.3448</td>
</tr>
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</table>

Normal distribution. Control was an untreated area on the opposite volar forearm. T0–T6 = Time points weeks 0–6.
also in the wash-out period of 2 weeks. Glycerol only had a significant effect on barrier function after 4 weeks (p = 0.0252). However, baseline values were lower in the glycerol group. ANOVA over time for both groups showed no significant effect: glycerol p = 0.2039; placebo p = 0.7845 (fig. 2).

The assessment of SC integrity and cohesion did not reveal any difference neither comparing the two groups nor over time (data not shown).

Reduction of Erythema
The glycerol-containing cream led to lower erythema values than the placebo cream after 2 weeks. After the end of treatment, erythema values in the glycerol group were still lower than in the placebo group. The differences between the two creams were however not statistically significant. ANOVA over time for both groups showed no significant effect: glycerol p = 0.7662; placebo p = 0.9202 (fig. 3).
Skin pH
A decrease in the early phase of the study was detectable for both formulations with a subsequent increase to the original values for both groups without a significant difference. ANOVA over time for both groups showed no significant effect: glycerol \( p = 0.7583 \); placebo \( p = 0.1371 \) (data not shown).

Reduction of SCORAD and Local Severity Values
No significant differences between the two treatment groups were detectable. Furthermore, no relevant changes over time could be recorded: ANOVA glycerol \( p = 0.9729 \); placebo \( p = 0.4116 \). The stable values could be partially explained by the small treatment area (volar forearm) in relation to the SCORAD assessment (total body; data not shown).

For estimation of local severity, a score was calculated based on the SCORAD system. At the beginning the values decreased (2.9/3.2 at the first visit to 1.5/2.4) under both treatments. In the placebo group, the values increased in the 4th week and decreased in the wash-out period. The differences between the groups were not statistically significant: ANOVA glycerol \( p = 0.1316 \); placebo \( p = 0.3782 \) (fig. 4).

Discussion
AD is a chronic, relapsing and pruritic inflammatory skin disease caused by complex interactions between multiple susceptibility genes and environmental factors [19]. The skin of patients with this disease shows a defective barrier as measured by TEWL, both in eczematous and clinically normal-appearing skin [20–22]. The daily skin care of patients with AD is – in addition to the treatment strategies with topical corticosteroids or calcineurin inhibitors – of great importance. The aim of the present study was to prove that a new glycerol-containing formulation for the topical therapy for patients with AD has a significantly better outcome than the same cream without glycerol. To evaluate the glycerol cream, we focused on its effects on capacitance, epidermal barrier function, erythema, skin surface pH and on its impact on the SCORAD and local severity. The concept of the study was to test the efficacy of emollients for AD in a drug-like phase III study.

The hypothesis was that the glycerol-containing cream has more positive effects than the glycerol-free product on the skin physiology of patients with AD. Changes in SC hydration appear to serve as an important biosensor function [23], and SC hydration is decreased in AD [24]. We could show that skin capacitance was significantly increased in glycerol-treated skin compared to placebo without glycerol. At all time points of the treatment, the difference between the two creams is statistically significant. In the 2 weeks after the end of the cream application (wash-out period), the corneometry values for glycerol remained higher than those for placebo indicating a sustainable effect beyond the cosmetic effect on dry skin of AD.

The mode of action of glycerol both on SC hydration and epidermal barrier function seems to be related to the aquaporin 3 channel. The basal layer of epidermal keratinocytes contains aquaporin 3, a small membrane protein that functions as a facilitated transporter of water and glycerol [25–28]. Glycerol is transported very slowly into the epidermis from the blood via aquaporin 3 channels [29], and thus its transport rate is sensitive to the intrinsic glycerol permeability of the basal keratinocyte layer. Mice deficient in aquaporin 3 have an approximately 3-fold reduced SC water content compared to wild-type mice, a reduced skin elasticity and delayed SC barrier recovery after tape stripping [29, 30]. The reduced SC hydration in aquaporin-3-deficient mice could not be corrected by skin occlusion or placement in a humectant atmosphere, indicating that water transport through aquaporin 3 is not a rate-limiting factor on SC hydration. Glycerol applied topically or systemically, but not glycerol analogues, corrected the SC hydration defect, reduced skin elasticity and delayed barrier recovery [25]. Thus, glycerol is a key molecule in skin physiology due to its humectant function and its effects of increased SC hydration. Patients with AD have a defective epidermal barrier function [31, 32]. As previously shown, glycerol is able to induce barrier recovery in an altered epidermal barrier in healthy subjects [8]. In the present study, lesional skin of atopic patients was investigated. Under such conditions we could detect a positive (but not significant) effect of glycerol for recovery of the altered epidermal barrier function. Loden et al. [33] were not able to detect differences in the biophysical assessment of epidermal functions in a placebo-controlled AD study but showed an improved clinical relief for urea. The erythema values as a marker of irritation or inflammation of skin were slightly lower in the group of patients treated with glycerol during the entire study.

The SCORAD values were assessed to get information on the severity of the AD of each patient. The value relates to the total body due to an estimation of the involved body surface area [18]. In this study, no significant differ-
ences between the two formulations were detectable. No relevant changes over time could be recorded. The stable values could be partially explained by the small treatment area (volar forearm) in relation to the SCORAD assessment (total body). In retrospect we state that it is not helpful to assess the SCORAD value for the evaluation of an only locally used formulation. The registration of the local severity score seems to be a suitable instrument for the assessment of local changes during treatment. This score was created to evaluate the local severity of AD at the forearm of the patients. The values of the local severity score decreased with fluctuations under the baseline values during the study duration in both treatment groups.

Concerning hydration, measured by capacitance of the SC, the glycerol cream has significant advantages compared to the glycerol-free formulation. A trend for better maintenance of barrier function, reduction of inflammation and decrease in local severity can be stated. With the group size in our study and the chosen study design we were not able to distinguish significantly between the glycerol-containing formulation and the control cream regarding improvement of epidermal barrier function, irritation parameters, surface pH and clinical scores. Since an improvement over time was detectable, we attribute this lack of difference to still excellent properties of the glycerol-free formulation. In our study no unpleasing sensations were reported by the patients during the treatment period. This is in accordance with data in the literature. There were no reports on adverse effects from glycerol, although it is used extensively [34]. Our findings support the use of glycerol in skin preparations as a humectant to increase the SC water content especially in AD. The benefit most likely derived from incorporating glycerol into a formulation designed for AD patients is the increase in SC hydration and the stabilization of the epidermal barrier function (even if the present study failed to provide statistical evidence for the latter effect). In the future, studies need to be conducted comparing and eventually combining glycerol-containing formulations with ceramide-containing creams. In conclusion, we state that the glycerol-containing cream is a safe product for daily skin care of patients with AD. This study demonstrates that it is possible to evaluate a product for daily skin care with a study protocol and with standards as for a fully registered drug.

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References


Glycerol and Placebo in Atopic Dermatitis


