Anti-Inflammatory Effect of a Novel Topical Herbal Composition (VEL-091604) Consisting of Gentian Root, Licorice Root and Willow Bark Extract

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ABSTRACT
The anti-inflammatory properties of the topical herbal composition VEL-091604 with gentian root, licorice root, and willow bark extract were assessed in a randomized, prospective, placebo-controlled, double-blind ultraviolet (UV)-erythema test study with 42 healthy volunteers in comparison to 1% hydrocortisone acetate. The efficacy and tolerability of VEL-091604 cream 2 times daily over 2 wk was evaluated in an open-label, prospective proof of concept study in 10 subjects with atopic dermatitis using a lesional SCORAD severity score. In the UV-erythema test VEL-091604 cream significantly reduced inflammation compared to placebo and was as effective as 1% hydrocortisone acetate. The clinical study with atopic subjects revealed a significant and rapid reduction of the lesional SCORAD severity score in the test areas after 1 and 2 wk. No adverse events were recorded. It is concluded that the herbal cream VEL-091604 with licorice root, willow bark, and gentian root extract display anti-inflammatory properties in vivo. It is a promising new treatment option for atopic dermatitis that warrants further investigation in controlled studies.

Introduction
Atopic dermatitis (AD) is a chronic inflammatory skin disease with a multifaceted pathogenesis, including genetic, immunologic, and skin barrier alterations [1]. Reduced expression of epidermal proteins such as filaggrin and reduced lipid synthesis results in an impaired epidermal skin barrier, eventually leading to dry skin, itching, and inflammation [2,3]. Acute inflammatory AD and severe or refractory cases usually require treatment with anti-inflammatory drugs. These include topical or systemic glucocorticoids for short-term intervention and systemic or topical treatment with calcineurin inhibitors such as cyclosporine A, tacrolimus, or pimecrolimus [4–6]. Very recently, the first monoclonal antibody targeting interleukin-4 has been approved for the systemic treatment of severe refractory AD [7].

Because the impaired epidermal barrier is considered a key pathogenetic factor in AD, current guidelines and therapeutic concepts of AD recommend topical therapy with barrier reconstituting emollients and natural moisturizing factors such as urea, amino acids, ceramides, and glycerin [4,8]. Some herbal extracts or isolated compounds have also been evaluated for their therapeutic potential in AD [9]. Limited controlled clinical studies have shown anti-inflammatory effects and therapeutic effectiveness in AD...
of St. John’s wort (*Hypericum perforatum*) [10, 11], tormentil (*Potentilla erecta*) [12], and licorice (*Glycyrrhiza glabra*) [13–15].

In the present paper, we assessed the anti-inflammatory effect of a topical herbal composition with licorice root (*Glycyrrhiza uralsis* Fisch. ex DC., Fabaceae), willow bark (*Salix daphnoides* Vill., Salicaceae) extract, and gentian root (*Gentiana lutea* L., Gentianaceae) extract, incorporated in a lipophilic oil/water emulsion. The study product VEL-091604 was assessed for its anti-inflammatory effect in a randomized, prospective, placebo-controlled, double-blind UV-erythema test with 42 healthy volunteers in comparison to 1% hydrocortisone acetate (HCA). The efficacy and tolerability of VEL-091604 cream was evaluated in a prospective, open-label proof of concept study in 10 subjects with AD. The subjects applied VEL-091604 cream 2 times daily in the morning and the evening for 2 wk, and the application compliance was checked by weighing the tubes before and at the end of the study. The clinical efficacy was evaluated using a lesional SCORAD severity score after 1 wk and 2 wk, respectively.

**Results**

In the UV-erythema test study, 42 volunteers were included. All subjects completed the study per protocol. The erythema of the test areas on the back of the volunteers was measured using a Mexameter (T0), and the test areas were then irradiated with 1.5 minimal erythema dose (MED). Subsequently, the test preparations were applied occlusively for 48 h. After a resting phase of 30 min, the test erythema areas were measured a second time (T1) and the erythema index T1–T0 was calculated. The reduction of the UV-induced erythema index by VEL-091604 cream was compared to the vehicle without actives and 1% hydrocortisone acetate (HCA). The efficacy and tolerability of VEL-091604 cream was evaluated in a prospective, open-label proof of concept study in 10 subjects with AD. The subjects applied VEL-091604 cream 2 times daily in the morning and the evening for 2 wk, and the application compliance was checked by weighing the tubes before and at the end of the study. The clinical efficacy was evaluated using a lesional SCORAD severity score after 1 wk and 2 wk, respectively.

In the open-label application study, 10 subjects with mild to moderate AD were included. The most frequent atopy parameters were pruritus (100%), dry skin (93%), and flexural eczema (88%). The subjects applied the study cream in the morning and the evening for 2 wk, and the application compliance was checked by weighing the tubes before and at the end of the study. All 10 subjects completed the study. The mean initial lesional SCORAD of the test areas was 9 (Fig. 2). The lesional SCORAD was significantly reduced after 1 wk, as well as after 2 wk (Fig. 2). An example of the clinical response to VEL-091604 cream is given in Fig. 3. Detailed information on the course of the partial SCORAD in each subject is given in Table 1.
studied compounds on skin cells [17]. Of the many constituents of licorice, the triterpenoids glycyrrhizin and glycyrrhetinic acid are the best viewes recently [17]. Therefore, glycyrrhizin was added to the herbal actives that mainly contain flavonoids and gentian such as gentiopicroside, sweroside, and swertiamarin which may exert antipruritic effects [31]. Other secoiridoids from gentian such as gentiopicroside, sweroside, and swertiamarin have been shown to possess wound healing and cytoprotective effects on fibroblasts [32], which may be of additional value in the topical treatment of AD.

The anti-inflammatory effect of VEL-091604 cream was confirmed in a 2-wk noninterventional open-label pilot study on atopic skin. VEL-091604 cream was applied 2 times daily over 2 wk in a defined test area and the lesional SCORAD was significantly reduced. It cannot be ruled out that the effect on atopic skin is mediated by the vehicle to some extent. However, since in the UV-erythema test the verum was significantly more effective than the vehicle, it is unlikely that the clinical improvement by VEL-091604 in the UV-erythema test the verum was significantly more effective than the vehicle, it is unlikely that the clinical improvement by VEL-091604 cream was simply an effect of the vehicle. To confirm the superiority of VEL-091604 in the clinical setting, either a vehicle controlled randomized study or a half-side comparison of vehicle and test area should be performed. However, the aim of the present proof-of-concept study was to confirm the anti-inflammatory effect that we actually observed in the UV-erythema test on atopic skin and to confirm the skin tolerance of VEL-091604 cream. In both the UV-erythema test and in the clinical study on atopic skin, no unwanted side effects were observed. It is hypo-

### Table 1 Characterization of subjects and test areas of the atopy study.

<table>
<thead>
<tr>
<th>Number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Atopy score</th>
<th>Localization and size (cm × cm) of test area</th>
<th>SCORAD day 0 (U1)</th>
<th>SCORAD day 7 (U2)</th>
<th>SCORAD day 14 (U3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>f</td>
<td>8</td>
<td>Hand (10 × 5 cm)</td>
<td>12</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>f</td>
<td>17</td>
<td>Abdomen (15 × 12 cm)</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>f</td>
<td>19</td>
<td>Forearm (8 × 10 cm)</td>
<td>14</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>f</td>
<td>13</td>
<td>Abdomen (8 × 8 cm)</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>m</td>
<td>14</td>
<td>Pretibial (12 × 5 cm)</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>f</td>
<td>19</td>
<td>Pretibial (7 × 5 cm)</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>f</td>
<td>17</td>
<td>Axilla (9 × 10 cm)</td>
<td>6</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>f</td>
<td>18</td>
<td>Abdomen (10 × 10 cm)</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>f</td>
<td>19</td>
<td>Bend of elbow (5 × 7 cm)</td>
<td>10</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>f</td>
<td>20</td>
<td>Hand (3 × 8 cm)</td>
<td>14</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>
esized that the putative mechanism of action of VEL-091604 cream is a combination of the 3 major active ingredients licorice root and glycyrrhizin, willow bark extract, and gentian root extract. Licorice root extract and glycyrrhizin display prominent anti-inflammatory effects [17–24]. Willow bark extract displays antioxidative and astringent effects [27, 28]. Gentian root extract possesses skin barrier reconstituting and immunomodulatory effects [29–32].

Taken together, the present studies confirm the anti-inflammatory effect of the topical herbal product VEL-091604 in vivo. The data presented here stimulate further clinical studies with VEL-091604 cream in AD.

Materials and Methods

Topical study products

The vehicle (placebo) cream and the verum (vehicle plus actives) of VEL-091604 was developed, produced, and provided by BSI Beaty Science Intelligence GmbH. The vehicle was an o/w emulsion with natural lipids and a hydrating aqueous phase. The composition of the vehicle according to the International Nomenclature of Cosmetic Ingredients was aqua, Helianthus annuus seed oil, alcohol, dicaprylyl ether, glycerin, Punica granatum seed extract, Butyrospermum parkii butter, cetearyl alcohol, cetearyl glucoside, glyceryl caprylate, xanthan gum, sclerotium gum, lysolecithin, sodium stearoyl glutamate, sodium anisate, sodium levulinic acid, Rosmarinus officinalis leaf extract, silica, pullulan, tocopherol, citric acid.

The actives of the verum cream VEL-091604 were as follows: a supercritical CO2 extract from G. uralensis root (0.10%) (batch no. 841106) with 6.8% licoricidin, 2.2% licorisoflavan, and 9.0% other isoflavans (Fig. 4) [25, 26]; dipotassium glycyrrhizate (Selco Wirkstoffe Vertriebs GmbH) (0.60%); a high pressure ethanolic spissum extract from S. daphnoides bark (0.25%) (batch no. 251502) with 12.3% gentiopicroside, 3.1% loganic acid, 0.8% swertiamarin, 0.4% loganin, 0.1% gentioside, 0.2% gentioside isomer, and 0.06% amarogentin (Fig. 6) (all from Flavex Naturextrakte GmbH). In the UV-erythema test, 1% HCA (Merck KGaA) incorporated in the vehicle was used as positive control.

The test preparations of the UV-erythema test (placebo, verum, and HCA as positive control) were provided in 5-mL blinded plastic tubes. In the AD application study, the VEL-091604 verum cream was provided to the subjects in 50-mL plastic tubes for application on the test area twice daily. The application compliance was checked by weighing the tubes before and at the end of the study.

UV-erythema test

The anti-inflammatory effect of VEL-091604 cream was evaluated with the UV-erythema test [33, 34]. The study protocol was approved by the ethics committee of the University of Freiburg (January 10, 2013; code 13/13) and written informed consent was obtained from all subjects. Inclusion criteria were healthy, nonsmoking persons of both sexes, age > 18 y, skin types II and III. Exclusion criteria were skin types I or IV, allergies, skin diseases, photosensitivity, sunbed tanning, metabolic diseases, use of any drugs (except contraceptives), alcohol consumption, infections, pregnancy, breast feeding, and participation in other studies during the last 2 mo. Forty-two volunteers aged between 22 and 64 y (42 ± 13 y) were included. Nine subjects were male (21.4%) and...
33 were female (78.6%). All subjects completed the study per protocol (no dropouts). The UV-erythema test was performed as described [33, 34]. In brief, after determination of the MED, the irradiation dose was individually calculated for each volunteer (1.5 MED). Background erythema (T0) was measured in all test areas before treatment using a Mexameter MX 16 (Courage & Khazaka Electronics GmbH) [33, 34]. The test areas were then irradiated with 1.5 MED. Subsequently, 50 µL of the test preparations were applied on the test areas using Finn chambers (1.8 cm², Almirall Hermal GmbH) and were fixed with Fixomull (6 × 4 cm, BSN Medical GmbH). After 48 h, the test substances were removed, and after a resting phase of 30 min, the test areas were measured a second time (T1) and the erythema index T1-T0 was calculated. As major readout parameter the reduction of the UV-induced ery-thema was determined.
thema by VEL-091604 cream was compared to the vehicle without actives and 1% HCA. Additionally, the skin tolerability of the test substances was determined by visual examination for the presence of erosions, edema, hemorrhages, vesicles, or pustules in the test area.

Noninterventional open-label application study

In the application study 10 subjects with mild to moderate AD aged between 4 and 55 y (mean age 29.5 ± 12.3 y) were included (Table 1). The study protocol was approved by the ethics committee of the University of Freiburg (March 24, 2016; code 608/15). The ethics committee approved to the inclusion of minor persons, and the minor persons agreed via written informed consent obtained from their parents. Written informed consent was also obtained from all other subjects. To assess the degree of atopy, the subjects filled a questionnaire recording the atopy criteria according to Diepgen et al. [35]. The most frequent atopy parameters were itching (100%), xerosis (90%), and flexural eczema (80%). The localization and size of the test areas is indicated in Table 1. Inclusion criteria were the presence of ≥ 4 atopy points [35] and reddened or eczematous skin with ≥ 2 points in the lesional SCORAD [12] of the test area. The following parameters were evaluated in the partial SCORAD: erythema, edema/papules, oozing/crusts, excoriation/lichenification, dryness/desquamation. The severity of these parameters was graded as follows: none = 0 points, slight = 1 point, moderate = 2 points, severe = 3 points. The mean initial lesional SCORAD of the test areas was 9 (Fig. 2), which corresponds to a moderate severity of eczema. Exclusion criteria were severe skin diseases, metabolic diseases, use of any drugs (except contraceptives), alcohol consumption, infections, pregnancy, breast feeding, participation in other studies during the last 2 mo, and known intolerance against ingredients of the test preparations. The study cream VEL-091604 was applied in the test area by the study participants or the parents of the minor aged 2 times daily in the morning and evening for 2 wk. The application compliance was checked by weighing the tubes before and at the end of the study. Study visits were at day 1 (U1), after 1 wk (U2), and after 2 wk (U3). At each visit, the test area was clinically evaluated using the lesional SCORAD. Additionally, the test areas were evaluated for newly formed erosions, edema, hemorrhages, vesicles, and pustules.

Biometrical analysis

In the UV-erythema test, the raw data (means of triplicate scans) of T0 and T1 measurements were processed electronically and were checked for correct data transfer and plausibility. The erythema index was calculated by subtracting the time zero values (T0) from the readings after 48 h (T1). The major readout parameter was the reduction of the UV-induced erythema. Statistical analysis was performed using the Wilcoxon test for pairwise comparisons. In the noninterventional application study the partial SCORAD data are shown. The readings were tested for significant differences between U1 and U2 and between U1 and U3, respectively, using the Wilcoxon test for pairwise comparisons. In both studies, the data are shown as box plots (Fig. 1 and 2). The box shows the median and the upper and lower quartile, which contains 50% of the data. The whiskers indicate the upper and lower extreme values. P-values are indicated in the figures by asterisks (p ≤ 0.05 = *; p ≤ 0.01 = **; p ≤ 0.001 = ***).

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Conflict of Interest

C. M. Schempp and U. Wölfle hold a patent on the topical application of bitter taste receptor agonists. The studies were performed without industrial funding.

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