Improvement of atopic dermatitis skin symptoms by *Vitreoscilla filiformis* bacterial extract

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease. The first line treatment of AD relies on the daily use of emollients to restore the skin barrier impairment associated with the disease. *Vitreoscilla filiformis* (Vf.) is a non photosynthetic bacterium and extracts of Vf. are endowed with properties which balance cutaneous immune-homeostasis. The aim of our study was to investigate the efficacy and safety of a 5% Vf. extract-containing ointment on mild to moderate AD in a randomised, double-blind, vehicle-controlled trial. Thirteen patients applied the treatment and the vehicle on symmetrical AD lesions (left versus right side of the body) twice daily for 4 weeks. The assessment of AD severity was done at each visit (Day 0, Day 14 and Day 28) using the modified eczema area and severity index (mEASI). Treatment with the ointment containing 5% Vf. extract significantly improved the AD skin symptoms. Beneficial effects were observed after two weeks of treatment and increased thereafter. These results suggest that Vf. extract could be favourably added to AD skin care emollients formulated for AD.

**Key words:** atopic dermatitis, bacterial extract, *Vitreoscilla filiformis*, mEASI, pruritus, double-blind study

**Material and methods**

**Preparation of Vf. bacterium extract**

V.f. is a non photosynthetic, non fruiting gliding bacterium belonging to the Beggiatoales order as defined in the Bergey’s classification [6]. Culture conditions may be briefly described as follows.

The culture medium is prepared with an autolytic yeast extract (2 g/L), a soya papainic peptone (2 g/L), glucose (3 g/L), Heller microelements (1 mL/L) and CaCl₂, 2H₂O (60 mg/L). Then the pH is adjusted at 7.20 before sterilization. After sterilization (121 °C/30’) the bioreactor medium is inoculated with V.f. (20 mM/L) issued of an Erlenmeyer batch culture. The batch step of the process is monitored on the following parameters: dissolved oxygen (> 10%), T° (28 °C) and pH (pH stat = 7.00). Due to its shear stress sensitivity, we used a draft tube agitation system to cultivate this strain. After 24–36 hours, the continuous culture step is engaged. According to the μmax (0.2 H⁻¹) the culture is controlled by the injection of fresh medium with a μ = 0.12 H⁻¹. In the same time we harvested equivalent...
volume of the whole culture in order to maintain the same culture volume in the bioreactor. The cells were separated from this continuous flow of the whole culture by centrifugation (10,000 g for 10 min). Finally, the biomass was stabilized by heat treatment (121 °C for 30 min). The reproducibility of the biomass is improved by this continuous production.

**Verum and vehicle ointments**

In order to demonstrate the efficacy of *V. f.* on AD skin inflammation we chose a standard base cream which contains a mixture of glyceryl mono/distearate and polyethylene glycol stearate, isoparaffin and cyclopentadimethylsiloxane. This base corresponds to an ointment not specifically designed for atopic skin. The vehicle was the base cream and the verum was prepared by mixing 5% of the *V. f.* biomass with the base cream.

**Study design**

The monocenter study was performed during autumn and early winter in order to avoid seasonal variations of the disease. The study involved a screening visit for patient selection and 3 visits for clinical examination at the start of the treatment (Day 0) then after 2 and 4 weeks treatment respectively. The ethics committee reviewed the protocol and granted approval of the study before its implementation.

**Patient selection**

Thirteen male or female AD patients aged 14 years and older (mean 35.7 ± 16.5) with a slight to moderate AD involvement of 5% to 60% of the total body surface area (BSA) were enrolled in the study. The main exclusion criterion was a serious skin disorder other than AD requiring treatment. Each patient (or parents for younger patients) gave written informed consent.

**Randomisation and blinding**

Each patient was assigned a number at baseline visit. For all patients, treatment was randomised according to body side (ointment containing 5% *V. f.* extract versus vehicle ointment). Both ointments (verum and placebo) were provided in tubes alike in all respects to safeguard blinding. Treatment boxes for each body side bore no information that might suggest they contained the study ointment or vehicle.

**Treatment**

At baseline, investigators defined symmetrical areas to be treated, gave the first ointment supply box, and explained the dosing procedure to patient. Treatment procedures consisted of applying a thin layer of ointment containing 5% *V. f.* extract twice daily to affected areas on one side of the body and applying the vehicle on the corresponding lesions on the contralateral side of the body. The following treatments were prohibited during the study: topical, inhaled, intranasal or systemic corticosteroids, antimicrobials, histamines, coal tar, topical non steroidal anti-inflammatory drugs, non steroidal immunosuppressants, UV light treatments, hypnotics, sedatives, other investigational drugs, bath oil and nonmedicated emollients.

**Assessment**

At baseline (Day 0), and after 2 and 4 week treatment, the investigator scored the following symptoms on each side of the body (right and left): erythema, edema-induration-papulation, excoriations, and lichenification using a scale 0 to 3 and estimated the percentage of total BSA affected by AD (0%-100%) in 4 body regions (head and neck, trunk, upper limbs, and lower limbs). Patients self-assessed the intensity of itching experienced during the 24 hours preceding clinical examination using a 10-cm visual analog scale, with 0 cm indicating “no itch” and 10-cm indicating “worst imaginable itch” at each side of the body (right and left). This assessment was used to calculate the modified eczema area and severity index (mEASI) at each side of the body (right and left). The mEASI is a variant of the eczema area severity index (EASI) developed by Hanifin et al. [8]. The mEASI is almost identical to the EASI but the former also includes an assessment of itching, considered as a primary symptom of AD [16]. Both EASI and mEASI have the advantage of including severity scores for individual symptoms of AD weighted according to the extent of affected BSA.

For each body region (head and neck, upper limbs, trunk, lower limbs) and each side of the body (right or left), the following steps were carried out: (a) an affected area score of 0 to 6 was assigned for the percentage of affected BSA (0%-100%); (b) the individual ratings for erythema, edema-induration-papulation, excoriations and lichenification were totaled (0 to 3 for each of the 4 symptoms); (c) the sum of individual symptoms (maximum = 12) was multiplied by affected area score (maximum = 6), with a maximum of 72; (d) since the patients were older than 7 years, the head and neck subtotal was multiplied by 0.1, the upper limb subtotal by 0.2, the trunk subtotal by 0.3, and the lower limb subtotal by 0.4 (e). All components were summed (maximum EASI = 72); and (f) the patient’s assessment of itching intensity was converted to an ordinal scale of 0 to 3 and then multiplied by the investigator’s total affected area score (0-6), with a maximum itching score of 18. The EASI was summed with the itching score, with a maximum mEASI of 90 (the sum of 72 and 18). This system of scoring AD is similar to the SCORAD index developed by the European Task Force on AD [9]. Investigators also assessed overall clinical improvement in the physician’s global evaluation of clinical response. “Cleared” indicated improvement by 100%, “excellent” by 90% to 99%, “marked” by 75% to 89%, “moderate” by 50% to 74%, “slight” by 30% to 49% and “no appreciable improvement” by 0% to 29% respectively. “Worse” indicated a worsening of the condition.

Adverse events were monitored on an ongoing basis. An adverse event was defined as any undesirable experience that occurred in a patient during the clinical trial, regardless of whether it was considered related to the product applied or not. Causally related adverse events were those assessed by the investigators as having a highly probable, possible, or not assessable relationship to the treatment product applied or where noted, adverse event data were presented irrespective of causal assessment.

**Statistical analyses**

The primary population was an intent-to-treat population, which comprised all patients who were randomised and
received at least one application of study ointment. The primary endpoint was mEASI. The evolution of mEASI, pruritus and affected body surface area from baseline to D14 and to D28 (end of the treatment) were analysed with paired non parametric tests (Wilcoxon signed ranks sum test) [10]. Due to the limited number of patients, asymptomatic results from inferential analyses could be misleading. Therefore, exact significance testing procedures based on two-sided non-parametric tests were performed. Statistical analyses were carried out using SPSS (SPSS Inc. Chicago, Illinois, release 11.0). The significance level was set at 5%.

Results

Efficacy
The study was conducted in 13 Caucasian AD patients (5 males and 8 females) aged 14 and older (mean age 35.7 ± 16.5). All patients had an active and extensive disease at baseline (in average one third of total body area affected by AD). Randomization resulted in a good matching between treatment sides. No statistically significant difference in the severity of AD between patient’s sides was observed at baseline. Patients applied the verum and vehicle on all symmetrical AD skin lesions.

AD elementary clinical symptoms were documented for each patient by mEASI. Comparison was done between the 5% Vf. ointment treated side and vehicle treated side (figures 1A and 2). Improvement in mEASI was observed in Vf. treated side at day 28 with a 42.9% decrease in mEASI, whereas vehicle treated side showed a decrease by 24.8% only (p = 0.008; Wilcoxon signed ranks test). Pictures of skin symptoms were obtained at selected sites during the course of the study. Representative patients are showed in figure 3.

Statistical analyses were also performed as secondary endpoints on the different parameters involved in the mEASI index including EASI, the pruritus severity index and the affected body surface area. There was a significant decrease

![Figure 1](image)

Figure 1. Clinical improvement of atopic skin symptoms. 95% CI delta treated versus vehicle in Modified Eczema Area and Severity Index (mEASI) (A), EASI (B), pruritus severity (C) and body surface area (constant A) (D). The mEASI considers the affected body surface area and the severity of erythema, edema, excoriations, lichenification (EASI) and itching. Y-axis unit represent delta values of the mean of EASI with their corresponding confidence interval (mean ± 2 SEM) and error bars represent 2SEM.
in EASI index in favour of the treated side at day 28 (p = 0.012; Wilcoxon signed ranks test) (figure 1B). The severity of pruritus was evaluated as a subjective measure of clinical efficacy. At baseline, no significant differences were noted between body sides (right or left). After 28 days of treatment, the level of pruritus was significantly decreased in the \textit{V. f.} treated side compared to the vehicle treated side (p = 0.046) (figure 1C). No significant difference in pruritus was observed at Day 14, as the affected body surface area at any assessment time (figure 1D).

**Tolerance**

The most common adverse events were pricking and burning sensations, with the same intensity in both sides and thus they were likely to be related to the vehicle. Three patients reported these events in the context of local discomfort. The incidence and intensity of these side effects shortly decreased and never lasted more than 15 minutes after topical application. Only a few sensations of dryness appeared (7%) on both treatment sides.

**Discussion**

In this study, the ointment containing 5\% \textit{Vf} extract was found to significantly alleviate the signs and symptoms of atopic skin in the study population when applied twice daily on all AD skin lesions compared to symmetrical AD lesions which received the placebo. It showed a relatively rapid onset of action, with beneficial effects observed after only 15 days of treatment and tending to increase thereafter. \textit{Vf} topical ointment was well tolerated, with most adverse events being localized, transient in nature and of moderate severity. These reactions of skin discomfort expressing as burning and stinging were observed with the vehicle and are to be related to the base formulation chosen to formulate the \textit{Vf} extract. Indeed, since emollients are part of the treatment of AD and could improve skin lesions when used alone we decided to test the efficacy of \textit{Vf} in a topical preparation devoid of strong emollient properties. Since the \textit{Vf} extract was able to improve atopic skin lesions despite the non-optimal formulation of the vehicle, we postulate that its effect would be better when formulated in an emollient specifically designed for atopic skin. Future studies will test this hypothesis.

Although \textit{Vf} extract was efficient in reducing the inflammatory signs of AD skin lesions, we do not know which components in the \textit{Vf} are responsible for the positive effects and by which mechanisms they improved the AD skin symptoms. The bacterial extract could contain some molecules able to restore the AD skin barrier impairment and therefore participate to the limitation of the penetration of pro-inflammatory environmental factors. Alternatively, some components of the \textit{Vf} bacterial extract could bear activities comparable to those found in cell walls of saprophyte bacteria from the gut [11]. Some unpublished studies have revealed that topical application of \textit{Vf} extract could decrease the intensity of arachidonic acid-induced skin inflammation and could down-regulate oxazolone-induced contact hypersensitivity. Moreover, unpublished studies have shown that \textit{Vf} extract promotes healing of epidermis and dermis in wound healing studies following suction blisters or skin incisions. Finally, a pilot clinical study has shown that the extract of \textit{Vf} includes moisturizing properties for the skin and protects skin against ultraviolet alterations.

While further investigations need to be done to more precisely define the mode of action of the \textit{Vf} extract, our results show that its incorporation into the formulation of emollients may help to reduce the AD skin symptoms.

![Figure 2. Modified Eczema Area and Severity Index (mEASI). At Day 28, change from baseline to the end of treatment for \textit{Vitreoscilla filiformis} treated side showed a 42.9\% decrease in mEASI whereas the vehicle treated side showed a decrease by 24.8\% (p = 0.008) (*). Y-axis unit represent the mean of mEASI and error bars represent 2 SEM.](image)

![Figure 3. Clinical presentation of patient 1 (A, B) and 7 (C, D), before (A, C) and at the end (B, D) of the treatment. \textit{Vf} extract is in the left in the figure.](image)
References