

CLINICAL AND INSTRUMENTAL EVALUATION OF THE ACTIVITY OF AN ANTI-WRINKLE COSMETIC PRODUCT ON CUTANEOUS RELIEF AND PHOTOAGED SKIN.

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Synopsis

The aim of this trial is to evaluate the efficacy of an anti-wrinkle cosmetic product on the cutaneous relief and condition of photoaged skin using a method of clinical scores together with an instrumental technique, namely image analysis of imprints taken from the crow's feet area of the eye. Thirty women presenting with cutaneous photoaging of the face applied the product twice daily for two months. Skin condition was evaluated before treatment (D0) and after 15, 30 and 60 days of treatment (D15, D30 and D60). Safety was verified at each visit. Progressive and significant improvement in cutaneous relief and the state of the skin was found, which was identical by both methods of measurement, thereby confirming the value of the clinical score method.

Riassunto

Lo scopo di questo lavoro è di valutare l'efficacia di un prodotto cosmetico anti-rughe sul rilievo cutaneo e lo stato della pelle foto-invecchiata, usando un metodo di valutazione clinica insieme ad una tecnica strumentale, ovvero l'analisi per immagini di impressioni prese dall'area delle "zampe di gallina" degli occhi. Trenta donne con segni di foto-invecchiamento cutaneo sul volto hanno applicato il prodotto due volte al giorno per due mesi. Lo stato della pelle è stato valutato prima del trattamento (D0) e dopo 15, 30 e 60 giorni di trattamento (D15, D30 e D60). La sicurezza è stata controllata durante ogni visita. È stato riscontrato un progressivo e significativo miglioramento nel rilievo cutaneo e nello stato della pelle, identico per tutti e due i metodi di misurazione, confermando quindi il valore del metodo di valutazione clinica.

INTRODUCTION

Cutaneous aging, which is the result of chronological aging plus actinic aging (1), is characterized by visible cutaneous damage, particularly on the face, which is regularly exposed to UV irradiation: dehydration of the epidermis reflects the slowing down of keratinocyte growth (2); the appearance of wrinkles, loss of firmness and suppleness in the skin result from the disorganisation of elastic fibres (3) and degeneration of collagen (4), which are components of the extracellular matrix synthesised by fibroblasts.

An extract of *Vitreoscilla filiformis*, a bacteria obtained from thermal sources and cultivated *in vitro* by biotechnology (5) induces multiplication of human keratinocytes cultured *in vitro* (C.M. Lapière et al, unpublished observations) and also has interesting effects on human fibroblast cultures: stimulation of cell proliferation and IL1 β production (J.A. Grimaud et al, unpublished observations). Furthermore, this bacterial extract induces cellular activation and the production of IL1 β in human macrophage cultures (V. Bayer et al, unpublished observations). IL1 β is involved in the regulation of elastin synthesis and in the mechanisms of dermal repair (6;7) and also acts on keratinocyte proliferation in the epidermis (8). In addition to its properties which have been demonstrated *in vitro*, *Vitreoscilla filiformis* probably induces, *in vivo*, restructuration of cutaneous tissues and thus improvement in the relief and appearance of the skin.

In this trial we have verified this hypothesis: the efficacy of a care product containing 1% *Vitreoscilla filiformis* extract has been studied over two months of treatment in a group of 30 women presenting with cutaneous photoaging of the face. The change in cutaneous relief and the state of the skin was followed using two distinct methods: clinical scores and image analysis of imprints taken from the crow's feet area of the eye; the correlation between the results obtained for cutaneous relief by the two methods has also been analysed.

METHODOLOGY

Volunteers

The trial was carried out in an open non-randomised fashion on 30 healthy Caucasian females, aged 38 to 60 years (mean age: 46 years). The volunteers had a normal skin and the state of their skin corresponded to degrees 3 to 5 on the photodigital scale described by Lamier and all (9). All the subjects gave their written informed consent in accordance with the ethics of cosmetic experimentation.

Product

The care product* was an oil/water cream containing 1% of the *Vitreoscilla filiformis* extract in association with traditional cosmetic active ingredients.

Treatment and monitoring of patients

The product was applied to the whole of the face, morning and evening, for 60 days. No other cosmetic product was allowed throughout the treatment period with the exception of cleansing products. This trial, carried out over a period of 2 months, included four control visits: before the beginning of treatment (D0), after 15 days (D15), 30 days (D30) and 60 days of treatment (D60). Evaluation of the state of the skin and cutaneous relief as well as verification of cutaneous safety were carried out by the investigator at each examination.

Evaluation methods

Clinical evaluations: scoring method (10)

The investigator carried out an analysis of skin condition (on the forehead, crow's feet, cheeks and medio-facial area) using a clinical score method. The following criteria were studied: cutaneous relief, suppleness, firmness, hydration, complexion. Each criterion, scored from 0 (negative value for the criterion) to 10 (positive value for the criterion) was evaluated individually. The means of the scores obtained on the four zones of the face were calculated for each criterion.

*Biotherm. Réducteur Rides. *Lifteur Rides Fermeté*

Instrumental methods: taking imprints and image analysis

Silflo replicas of cutaneous relief (11) were taken from a precisely delineated zone including the crow's feet area. These imprints were quantitatively analysed by image analysis (12), using a video camera (Cohu) coupled to a microcomputer and using image analysis software (Quantrides, Monaderm). This technique enabled the number of wrinkles per unit surface area (u.s.) to be quantified as well as their depth (μm).

Subjective evaluation

After 4 and 8 weeks of treatment, the subjects evaluated the activity of the product on their wrinkles (satisfactory or unsatisfactory with respect to the results obtained), scoring the firmness, softness and hydration of the skin, using a scale of 0 to 4 for each criterion (0: unsatisfactory; 4: satisfactory).

Statistical analysis

A two-tailed Student's *t* test for paired series was used to analyse the differences between the values obtained before treatment and after 15, 30 and 60 days of treatment (clinical scores and image analysis). The differences were considered significant when $p < 0.05$. The correlation coefficient *r* and its threshold of significance *p* were calculated by linear regression analysis using the means of the values obtained for all the subjects at each control vi-

sit (D0, D15, D30, D60), in order to determine the correlation between the results recorded by the scoring method and those obtained by image analysis.

RESULTS

Clinical change

The mean of the scores obtained for all the subjects (\pm standard deviation from the mean), at each control visit and for each criterion, is given in Figure 1. A progressive and significant improvement in cutaneous relief can be observed during treatment. In comparison to D0 (before treatment), the cutaneous relief improves by 13, 34, and 57% after 15, 30 and 60 days of treatment (see Table 1).

The treatment also induces a significant increase in the firmness of the skin and an improvement in suppleness, hydration and complexion.

Quantitative change in cutaneous relief

The effect of treatment on the number and depth of wrinkles was determined by image analysis of the imprints. Figure 2 gives the means of the measurements made for all the subjects. The number of wrinkles is reduced in a significant fa-

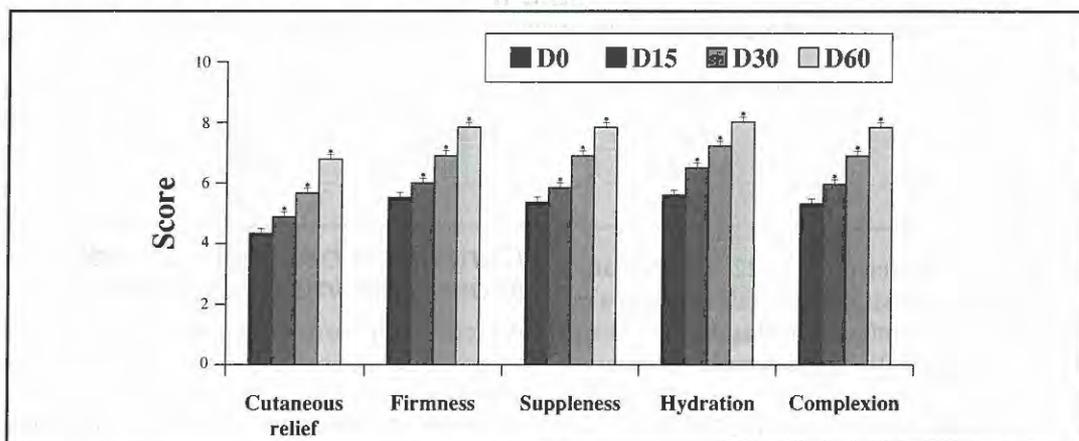


Fig 1: Clinical scores (mean \pm standard deviation from the mean).

D0: Before treatment; D15: After 15 days of treatment; D30: After 30 days of treatment; D60: After 60 days of treatment.

* significant difference with respect to D0 ($p < 0.05$).

Subjective evaluation

Figure 4 gives the evaluation made by the subjects which confirms the activity of the product in terms of wrinkle improvement (90% of subjects were satisfied at D30). Good activity of the product on firmness, softness and hydration of the skin was also recorded.

Product safety

The use of the product did not lead to any unwanted cutaneous reaction, demonstrating its complete local safety.

DISCUSSION

The results of this trial demonstrate the efficacy of the studied product in terms of improvement in relief and the characteristic signs of photoaged skin, which are seen beginning from the 15th day of treatment onwards. This improvement continues to the end of treatment. The change in the clinical scores for cutaneous relief is correlated to a reduction in the number and depth of wrinkles measured by image analysis. This correlation shows the reliability and value of clinical scores for evaluation of cutaneous relief.

It therefore seems that the activity of *Vitreoscilla filiformis* measured *in vitro* on cutaneous cells has also been verified *in vivo*: activation of cutaneous cells by the bacterial extract may lead to an increase in keratinocyte cohesion as well as an increase in connective tissue density, leading to an improvement in cutaneous relief and the state of the skin.

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