Whitening effect of a dermocosmetic formulation: a randomized double-blind controlled study on melasma

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Received 31 October 2005, Accepted 29 December 2005

Keywords: melanin, corneomelametry, ULEV method, skin lightening, spectrophotometry, colour

Synopsis

Melasma is an endocrine-mediated facial hypermelanosis with epidermal and occasionally dermal components. We tested in a randomized double-blind design the effect of a whitening formulation (Thiospot intensive®) on this skin disorder. The product containing ethyl linoleate, thioctic acid, octadecenedioic acid, lactic acid and ethylhexyl methoxycinnamate was applied twice daily for 3 months by 20 young women. Another control group of seven women received a non-skin lightening formulation. Clinical assessments were made at 1-month intervals. In addition, objective measurements of the hypermelanosis were performed using narrow-band reflectance spectrophotometry, image analysis of video-recorded ultraviolet light reflection (ULEV method) and photodensitometry of the corneomelametry test. A significant lightening effect was evidenced beginning the second month of treatment with the whitening formulation. No significant effect was observed with the control product.

Résumé

Le mélasma est une hypermélanose faciale hormono-dépendante qui a des composantes épidermiques et parfois dermiques. Nous avons testé en double insu et randomisation l'effet d'une formulation dépigmentante (Thiospot intensive®). Le produit contenant de l'éthyl linoleate, de l'acide thioctique, de l'acide octadecenedioïque, de l'acide lactique et de l'éthylhexyl mèthoxycinnamate a été appliqué deux fois par jour pendant 3 mois chez 20 jeunes femmes. Un autre groupe témoin de 7 femmes a reçu une formulation placebo. Des évaluations cliniques ont été réalisées à un mois d'intervalle. En complément, des mesures objectives de l'hypermélanose ont été réalisées en utilisant la spectrophotométrie en bande étroite par réflectance, l'analyse d'images d'enregistrement video de la réflexion de lumière ultraviolette (méthode ULEV) et la photodensitométrie du test par cornéomélamétrie. Une réduction significative de la mélanose a été mise en évidence dès le deuxième mois de traitement par la formulation dépigmentante. Aucun effet significatif n'a été observé avec le produit contrôle.

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Introduction

The innate melanin pigmentation of skin is modulated during lifetime by a series of factors including specific neuromediators, hormones and cytokines. Ultraviolet light, chronic inflammation and rubbing of the skin are some of the most important triggering factors. Melasma is an hormone-dependent hypermelanosis of the face. It represents a stubborn problem for young women because this condition has a multi-pronged origin and the treatments are lengthy and not always successful [1]. In this sun-sensitive condition, melanin can be in excess inside the epidermis and/or released inside the superficial dermis. Skin lightening products which are numerous on the market [2–4] represent the most frequent therapeutic option for melasma. When active *in vivo*, the whitening products can decrease the melanin production or its transfer to the keratinocytes, thus limiting the

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contribution of the epidermis to the hypermelanosis. By contrast, the dermal melanophages remain unaffected.

The objective assessment of pigmentary changes benefits from a series of distinct sensitive methods [5, 6]. Among them, the narrow-band remittance spectrophotometry yields the melanin index M, assessing the content of skin melanin [4, 7–10]. Any discrete hyperpigmentation can also be revealed under long-wave ultraviolet light illumination. The ultraviolet light-enhanced visualization (ULEV) method increases the contrast between the faint or almost invisible hypermelanosis and the surrounding skin. This increased contrast is the result of the greater reflection of ultraviolet wavelengths than visible light by collagen, and their absorption by melanin [4, 11–16]. Corneomelametry (after corneocyte, melanin, metry) is another objective method designed to quantify the melanin content of corneocytes in the stratum corneum [4, 13]. For that purpose, cyanoacrylate skin surface strippings (CSSS) are collected, stained using argentaffin histochemistry and submitted to quantitative photodensitometry under the microscope [13].

The aim of the present study was to assess the whitening effect of a composite whitening formulation on melasma.

Volunteers and methods

The trial was performed in accordance with the Declaration of Helsinki and its subsequent amendments. The patients gave their informed consent after the whole procedure of the study had been fully explained. A total of 27 healthy phototype III women were enrolled in this study conducted in the spring season in Liège. They were aged between 27 and 38 years and presented typical melasma of the forehead for at least 6 months. Hyperthyroidism was not detected in any of the volunteers. They were randomized to receive in a double-blind design either a composite whitening product (Thiospot intensive®; General Topics, Salo, Italy) or a non-skin lightening skincare formulation (Eucerin®, Beiersdorf, Hamburg, Germany). The whitening formulation contained a mixture of ethyl linoleate, thioctic acid (α-lipoic acid), octadecenedioic acid, lactic acid and ethylhexyl methoxycinnamate. All volunteers were under oral contraception but received no other drug. The products had to be applied twice daily on melasma for 3 months.

Clinical and biometrological assessments were performed at monthly intervals. They corresponded to overall visual pigmentation gradings on a 4-level linear scale (0: absent, 1: discrete, 2: moderate, 3: intense). Three complementary assessments were performed using the Mexameter® MX16 (C + K Electronic, Cologne, Germany), the Visioscan® VC98 (C + K electronic) and the corneomelametry test, respectively.

The Mexameter was used as previously described [7, 8, 13] to derive the M index of the target site on the mid-forehead. Three measures were taken and the median value was recorded. Visioscan® VC98 recordings were also made on three contiguous fields of the same area of the forehead. Each of them measuring 1×0.8 cm was digitalized in a 512×400 pixels frame. Image analysis of the epidermal hypermelanosis was performed as previously described [11–16]. In addition, corneomelametry was performed from the same sites for assessing the melanin load inside the stratum corneum. For this purpose, CSSS were harvested from the target site. They were stained by Masson's argentaffin method. The samples were placed under a photomicroscope equipped with an internal photodensitometer. The recorded values which increased with the staining intensity were an estimate of the melanin load inside corneocytes.

Statistical analysis

An intent-to-treat analysis was performed. Absolute values of each parameter were recorded and their means and SD were calculated at each evaluation time. The two-tailed paired *t*-test was used to compare the data both at inclusion and at completion of the study. Variance analysis was performed to assess the changes occurring in time. A P value lower than 0.05 was considered significant. Because of interindividual differences at inclusion, percentage variations form baseline values were also computed to describe the relative changes in time.

Results

At baseline, the clinical rating of the melasma pigmentation of the women receiving the whitening product was rated 2.60 ± 0.50. This corresponded to a moderate to severe hypermelanosis. A significant reduction to 1.95 ± 0.69 ($P < 0.05$) was observed after 2 months of treatment. It

corresponded to a 25% improvement leading to moderate hypermelanosis. A further reduction down to 1.65 ± 0.67 ($P < 0.001$) was reached after 3 months of treatment. This value corresponded to a 36% improvement and to a mild to moderate hypermelanosis.

At inclusion, the clinical pigmentation rating was 2.71 ± 0.49 in the volunteers receiving the non-skin lightening product. No significant changes were yielded during the next 3 months. The severity in clinical melanosis in both groups of women was similar at inclusion. At the end point, the group having received the whitening product had a skin significantly lighter ($P < 0.01$) than the control group.

The value of the M index progressively decreased during treatment by the whitening product (Table I). The reduction reached significance ($P < 0.001$) after 2 (−10%) and 3 months (−19%). No significant lightening effect was observed in the control group.

The intra-epidermal melanin quantification by the ULEV method revealed a significant reduction ($P < 0.001$) after 2 months (−29%) and 3 months (−44%) of treatment with the whitening product (Table I) (Fig. 1). By contrast, no significant changes were observed in the control group.

The amount of melanin in the stratum corneum as assessed by corneomelametry decreased significantly after 2 months (−10%, $P < 0.01$) and 3 months (−21%, $P < 0.001$) of treatment with the whitening product (Table I). No significant changes were yielded in time in the control group.

Discussion

Regulation of melanin synthesis depends on multistep enzymatic processes [4, 13, 17]. The current strategies for developing new compounds controlling the excess in eumelanin pigmentation target several of the biological mechanisms. The main targets include: (a) the levels of expression of

Table I Biometrological assessments of the lightening effect

	Melanin index (AU)		ULEV method relative area (%)		Corneomelametry photodensitometry value (AU)	
Evaluation time	Placebo	Treated	Placebo	Treated	Placebo	Treated
Baseline	484 ± 15	492 ± 15	60 ± 8	55 ± 11	127 ± 11	130 ± 12
1 month	484 ± 13	482 ± 21	62 ± 8	52 ± 9	129 ± 7	124 ± 13
2 months	476 ± 24	443 ± 26***	59 ± 6	39 ± 10***	127 ± 11	117 ± 12**
3 months	474 ± 19	397 ± 31***	58 ± 8	31 ± 10***	124 ± 8	103 ± 17***

Data are expressed as mean ± SD. AU: arbitrary units. Differences from baseline: *$P < 0.05$, **$P < 0.001$, ***$P < 0.001$.

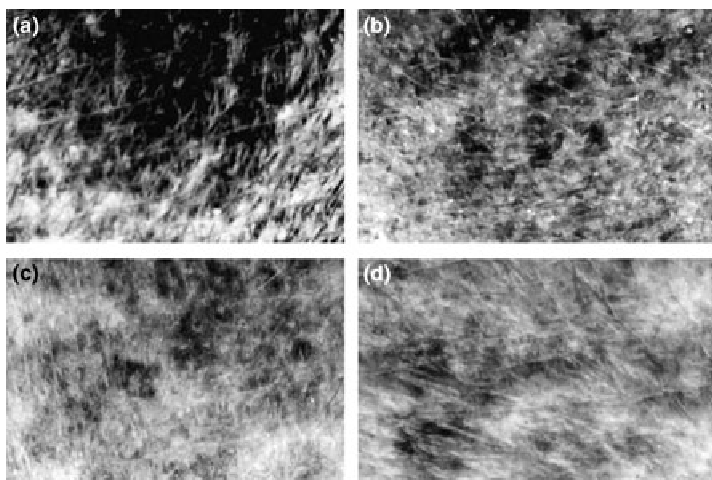

Figure 1 Evolution of the ULEV aspect of melasma during treatment with the whitening product: (a) inclusion; (b) after a 1-month treatment; (c) after a 2-month treatment; (d) after a 3-month treatment.

tyrosinase and of the tyrosinase-related proteins TRP-1 and TRP-2; (b) thiol conjugation (e.g. with glutathione or cysteine) leading to the formation of phaeomelanins; (c) the α-melanocyte-stimulating hormone (α-MSH) and its receptor which is coupled to the adenylate cyclase/protein kinase A pathway; (d) the product of the agouti locus; (e) the regulation of melanosome formation and clumping; (f) the melanocyte dendritogenesis, and (g) the mechanisms regulating the uptake and distribution of melanosomes in recipient keratinocytes [4, 11, 18–20]. Any interference at these levels decreases the amount of melanin in the epidermis and/or contributes to the formation of various forms of melanins with different light absorption characteristics [13].

The development of novel topical hypopigmenting products benefits from *in vitro* technologies including enzymatic, cell-based and engineered tissue assays [21]. Unfortunately, the extrapolation to the *in vivo* efficacy may remain unsatisfactory [13]. In addition, most animal models for hypopigmenting efficacy are quite often inadequate [2]. Hence, clinical trials performed in human volunteers are critical to determine the efficacy profile of these topical products. The objective assessment of the extent and severity of skin pigmentation disorders benefits form a series of non-invasive methods [11–13].

In this study, we used four complementary methods. The global assessment of melasma was rated by visual inspection. The specific melanin-dependent colour was measured by a narrow-band reflectance spectrophotometer. The melanin content was derived from the M index representing the changes in wavelengths of the light emitted by the diodes of the device and those remitted by the skin [5]. This method did not distinguish the epidermal from the dermal melanin location. The ULEV method brought another information as only the epidermal melanin load was concerned in the quantification. Corneomelametry assisted by photodensitometry is another quantitative method to access the load in argentaffin compounds present inside the superficial layers of the stratum corneum. The sensitivity of the panel of these complementary methods is indisputable improving the objectivity in comparative assessments.

Most commercial whitening cosmetics, over-the-counter products and dermatological formulations have shown temporary reduction in melanin production and accumulation in the epidermis. The presently tested formulation showed *in vivo* efficacy while on treatment.

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