Original Contribution

Facial skin-lightening benefits of the tetrapeptide Pro-Lys-Glu-Lys on subjects with skin types V–VI living in South Africa

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Summary

Background Irregular skin pigmentation may be a substantial contributor to the signs of aging and to a person's lack of psychological well-being. Although a large number of skin-lightening agents are available, the opportunity exists to identify more efficacious agents, agents that target alternative biological mechanisms.
Aims To provide clinical evidence of the skin-lightening effect of the tetrapeptide, Pro-Lys-Glu-Lys (PKEK), on subjects with skin types V–VI living in South Africa.
Methods Pro-Lys-Glu-Lys was evaluated in a double-blind and vehicle-controlled clinical study using expert grading of digital images by comparing its effects in subjects with skin types V–VI suffering from facial melasma and postinflammatory hyperpigmentation.
Results This study demonstrated the efficacy of PKEK on subjects with skin types V–VI. On comparing the two treatments, the skin-lightening peptide-containing formulation was significantly superior to the vehicle at 12 weeks on overall appearance ($P < 0.05$) and evenness of skin tone ($P < 0.01$).
Conclusions The tetrapeptide, PKEK, has proven skin-lightening benefits on skin discoloration from melasma and postinflammatory hyperpigmentation. These studies have been conducted on subjects with skin types V–VI living in South Africa, but we believe this technology to be suitable for all racial groups.

Keywords: α-melanocyte-stimulating hormone, peptide, photoaging, proopiomelanocortin, proline attached to its N-terminus, skin-lightening

Introduction

All of us experience the signs of aging such as wrinkles, solar lentigos and homogeneity of skin coloration.[1] Age spots are common in Asian and Caucasian subjects, and unevenness of skin coloration together with postinflammatory hyperpigmentation can occur in the darker skin phenotypes, although not exclusively.[2] Postinflammatory skin hyperpigmentation can occur especially after having acne. Melasma, which affects predominantly women, is also a relatively common disorder of macular skin hyper-pigmentation and is especially prevalent in subjects with darker complexions.[3,4]

Skin pigmentary disorders can have profound effect on a person's quality of life and can significantly affect a person's psychological well-being.[5–7] Indeed, consumer research indicates that we are still not fully meeting consumer expectations even with the best available skin-lightening treatments.[8] As a result, products that help to control the aberrant skin hyperpigmentation are in high

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demand.[9] Traditional routes include the use of sunscreens, but inhibition of melanocyte activity, reduction in melanosome transfer and dendricity together with the accelerated removal of melanin by inducing epidermal proliferation are typical.[9] Typical actives include retinoids,[10–12] niacinamide,[13–16] kojic acid,[17] arbutin and derivatives,[18,19] bisabolol,[20] soybean trypsin inhibitors,[21] azelaic acid,[22] licorice derivatives such as Glabrene and isoliquiritigenin,[23] vitamin C derivatives and other antioxidants,[24] N-acetyl glucosamine,[25,26] N-undecy-10-enoyl-L-phenylalanine,[27] hydroxyacids [28,29] and octadecenedioic acid.[30–32]

To develop new potential ingredients for skin-lightening products, we have focused on the inflammatory pathways involved in skin pigmentation.[33] Recently, it has been shown that a peptide comprising Lys-Glu-Lys (KEK), which was further modified with a proline attached to its N-terminus (PKEK) to stabilize its structure, reduces interleukin-6 and 8, tumor necrosis factor-α and cyclo-oxygenase gene expression in ultraviolet (UV) light-stimulated keratinocytes *in vitro* and *in vivo*.[34] One of the proopiomelanocortin (POMC)-derived peptides, α-melanocyte-stimulating hormone (α-MSH), was also induced by UV irradiation from both keratinocytes and melanocytes and was in addition responsible for inducing melanogenesis. PKEK was equally capable of reducing POMC in UV-irradiated keratinocytes and in skin. Thus, PKEK interferes with the keratinocyte to melanocyte signaling processes involved in skin pigmentation was shown to reduce tyrosinase expression *in vivo*.[34]

The purpose of this study was to evaluate the skin-lightening activity of PKEK clinically on subjects with skin types V–VI suffering with facial melasma and postinflammatory skin hyperpigmentation owing to previous experience of acne.

Material and methods

All studies complied with the Guidelines for Medical Experiments in nonpatient human volunteers published by the Association of the British Pharmaceutical Industry and the World Medical Association's Declaration of Helsinki (2000) concerning biomedical research involving human subjects and the guidelines for good practice in the conduct of clinical trials in human participants in South Africa. Each subject received written informed consent and a copy of the INCI ingredient list for each product. All studies were performed in compliance with good clinical practice. The study was approved by the University of Limpopo campus research and ethics committee. The subjects were instructed not to use any cosmetic products including topical skin-lightening agents, hydroxyacids and retinoids for a washout period of 2 weeks preceding the studies. Use of any product other than the test products provided was not allowed for the duration of the studies.

Double-blind full face, randomized and vehicle-controlled study of the effects of a skin-lightening peptide (PKEK) on facial skin of subjects with skin types V–VI

Fifty healthy female volunteers aged between 18 and 50 years (38.4 years ± 10.5) suffering from hyperpigmentation from mild acne and melasma were enrolled in this 12-week double-blind and placebo-controlled clinical study. The formulations with or without PKEK were in emulsions consisting of C12-15 alkyl benzoate, PPG-3 myristyl ether, polyglyceryl-3-methylglucose distearate, glyceryl stearate, stearyl alcohol, and preservative. During the study, a skin examination of the test areas was performed under controlled lighting conditions and skin examinations were visually evaluated at baseline, 4, 8 and 12 weeks.

Evenness of skin tone and overall skin appearance was compared using two separate numerical grading scales. For evenness of skin tone, the skin was assessed using a five-point grading scale ranging from a grade of 1 being no contrast of lesions vs. surrounding skin and a grade of five being very high contrast for the skin tone (none = 1, slight = 2, moderate = 3, high = 4, very high = 5).

For skin appearance, a 5-point grading scale was also used with grade 1 being very bad on overall skin appearance and a grade of 5 being very good for the skin appearance (very bad = 1, bad = 2, moderate = 3, good = 4, very good = 5).

Photographs were taken using the Visia-CR booth (Canfield Scientific Inc., Fairfield, NJ, USA) equipped with a high-resolution Nikon digital camera (Nikon D200, Nikon Corporation, Tokyo, Japan) using the Photo Tools Software of the right and left oblique together with front views of the face of each panelist. Subject facial distance and positioning is controlled via a fitted head and chin rest. For each subject, the baseline image was taken as a guide for repositioning the subjects head in subsequent time points by superimposing the two images. All images were taken with the same equipment under the same conditions (lighting, distance, head position, etc.) at all time points. A standard photographic color card was included to check for color reproducibility. These photographs were rated by an expert blinded assessor on evenness, background skin color, contrast between the background skin color and the lesions, the number of

lesions and overall appearance. The grader was a trained clinical assessor with over 20 years of experience in assessing facial pigmentation in these skin types. The study period was February to May. Subjects applied approximately 0.5 g of product per application.

Statistical analysis

All data were collected in Microsoft Excel 2003 before transferring to Graphpad prism (5.00 for Windows; GraphPad Software, San Diego, CA, USA). Data were checked for normality using the D'Agostino and Pearson omnibus normality test, and as the data set was not normally distributed, it was compared with the nonparametric Wilcoxon signed rank test. Values are expressed as arithmetic mean ± standard error of the mean (SEM). P values <0.05 were considered significant.

Results

Double-blind full face, randomized and vehicle-controlled study of the effects of a skin-lightening peptide (PKEK) on facial skin of subjects with skin types V–VI

The overall appearance and evenness of skin tone statistically improved from baseline for the skin-lightening peptide-treated skin (Fig. 1). For overall appearance, this was as early as 2 weeks into the study, whereas for evenness of skin tone, this only occurred after 4 and 8 weeks for the number of lesions. The vehicle also influenced the overall appearance and evenness of skin tone. Nevertheless, on comparing the two treatments, the skin-lightening peptide-containing formulation was significantly superior to the vehicle at 12 weeks on overall appearance ($P < 0.05$) and evenness of skin tone ($P < 0.01$). The improvement in overall appearance and skin tone was approaching 2 units from baseline and 1 unit difference compared with the vehicle. Examples of the efficacy of PKEK are shown in Fig. 2. These results demonstrate the effect of the skin-lightening peptide improving evenness of skin tone and overall appearance on facial skin in subjects with skin types V–VI. In this study, however, no distinction was made between the causes of the hyperpigmented lesions.

Discussion

Age-related changes in visible skin condition are driven primarily by an increase in uneven skin pigmentation (derived from both melanin and hemoglobin) as well as changes in skin surface topography.[1,35] Recent research

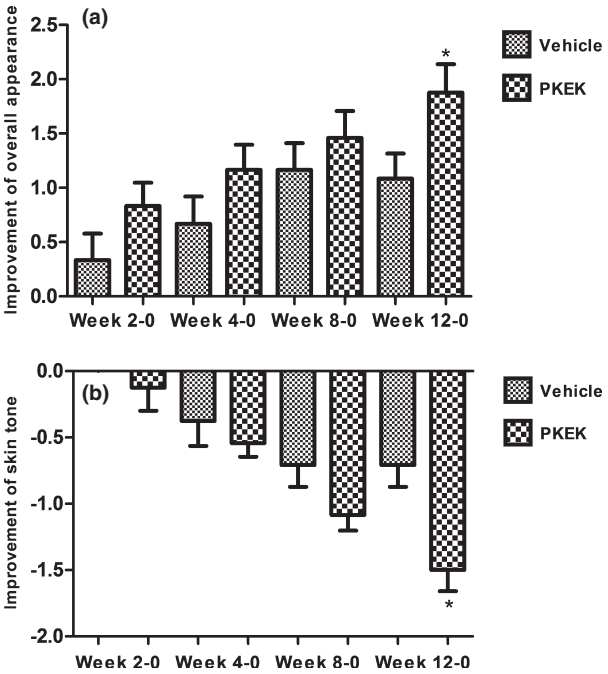

Figure 1 Effect of PKEK vs. vehicle on improvement from baseline of (a) overall skin appearance, a 1–5 grading scale was used of grade 1 being poor overall appearance and 5 being very good. A positive score indicates an improvement in overall skin appearance. (b) skin tone of African subjects, a 1–5 grading scale was used of grade 1 being no contrast and grade 5 being high contrast between the lesions and the surrounding skin. A negative score indicates an improvement in skin contrast. Both assessments were significantly different to the vehicle at 12 weeks $^{*}P < 0.05$.

by Fink *et al.* [36] revealed that humans are sensitive to the visible signs of cutaneous aging by demonstrating that variation in skin color distribution affects age, attractiveness, and health perceptions of female faces. Those authors found that skin color distribution alone, independent of skin surface topography and facial shape, accounted for a span of up to 20 years of age perception.[37] Conversely, they also established that a positive correlation of visual attention and attractiveness judgment exists with skin color evenness. Female faces with more even skin coloration received higher visual attention and were considered younger and more attractive.[38–40]

UV irradiation is clearly driving the problems with unevenness of skin color. Upon exposure of the skin to UV radiation, melanogenesis is enhanced by the activation of the key enzyme of melanogenesis, tyrosinase.[9] There are many effectors of this stimulation, including cytokines, such as interleukins and TNF-α, and growth factors. However, there are a number of keratinocyte

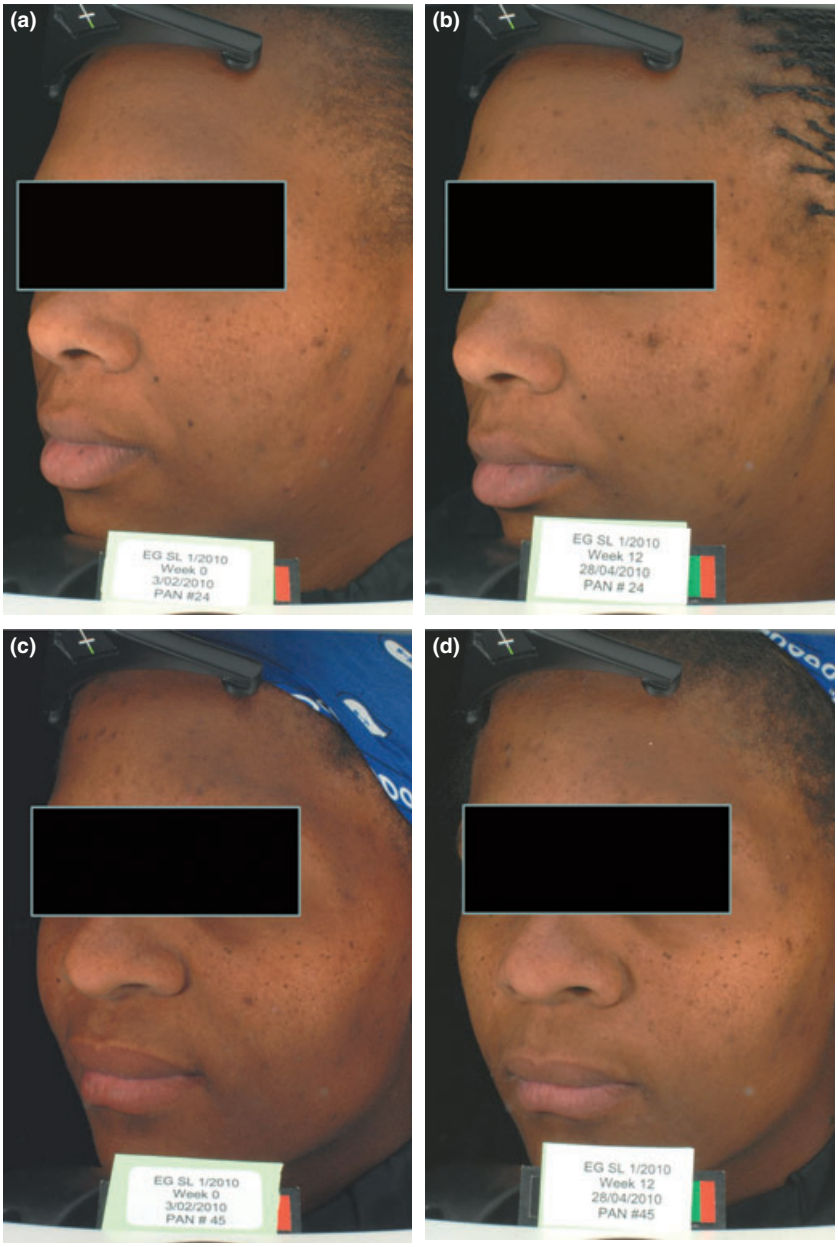

Figure 2 Representative photographs of the typical efficacy of PKEK at 12 weeks of treatment. (Vehicle a,b & PKEK c,d). The PKEK formulation can be seen to improve skin tone and overall skin appearance.

paracrine stimulators of melanogenesis such as endothelin-1, stem cell factor, prostaglandins, catecholamines and other cytokines and growth factors. POMC-derived peptides (α-MSH, β-MSH, ACTH) are also paracrine mediators of melanogenesis.[9] The pivotal effect of these hormones on melanogenesis has been demonstrated *in vivo* where a systemic administration of α-MSH, β-MSH, or ACTH increases skin pigmentation, predominantly in sun-exposed areas.[41] The POMC peptides exert their effects through a cyclic adenosine 3′,5′-monophosphate (cAMP)-dependent mechanism. On binding to the G-protein-coupled receptor melanocortin receptor 1 (MC1R) and activating adenylyl cyclase (AC), the production of cAMP consequently stimulates protein kinase A (PKA), which then phosphorylates enzymes, ion channels, and several

regulatory proteins eventually leading to a stimulation of the melanogenic pathway. Regulation of transcriptional activity by activated PKA involves phosphorylation of the cAMP-responsive element-binding protein (CREB) and activation of the microphthalmia-associated transcription factor (MITF). In turn, MITF efficiently activates the melanogenetic enzyme genes, such as tyrosinase and TRP-1/TRP-2.[9]

Typical actives include retinoids,[10–12] niacinamide,[13–16] kojic acid,[17] arbutin and derivatives,[18,19] bisabolol,[20] soybean trypsin inhibitors,[21] azelaic acid,[22] licorice derivatives such as Glabrene and isoliquiritigenin,[23] vitamin C derivatives and other antioxidants,[24] N-acetyl glucosamine,[25,26] N-undecy-10-enoyl-L-phenylalanine,[27] hydroxyacids [28,29] and most recently octadecendioic.[30–32]

Of these, N-undecy-10-enoyl-L-phenylalanine has a mechanism related to PKEK activity.[27] It has been proposed that phenylalanine is critical in the interaction of peptide ligands with the alpha-MSH receptor, and phenylalanine-containing peptides have the potential to activate or inhibit its action.[42–44] N-undecy-10-enoyl-L-phenylalanine has been reported to be an alpha-MSH antagonist and a skin-lightening agent that has additional activity in the presence of niacinamide.[25,26]

We took a different approach and evaluate a peptide that had the potential to down-regulate the expression of inflammatory cytokines as well as POMC.[34] POMC has been shown to undergo age-related changes in primary keratinocyte cultures from human epidermis.[45] Thus, we are targeting the expression of genes involved in the melanogenic stimulus. Indeed, those studies demonstrated both *in vitro* and *in vivo* that PKEK down-regulated many keratinocyte paracrine stimulatory molecules, including POMC. As a result, we were interested in the clinical effects of PKEK on hyperpigmentary skin problems.

The study we conducted was blinded expert skin grading of photographs taken from subjects with skin types V–VI who were suffering from melasma and postinflammatory hyperpigmentation resulting from previous acne. Both the vehicle- and the PKEK-containing formulation improved the evenness of skin coloration and overall skin appearance compared with the baseline skin condition, but the PKEK treatment was found to be superior and significantly different to the vehicle after 12 weeks of treatment. There have been many studies examining the effect of agents on melasma and the efficacy ranged from approximately 15–70% improvement using the Melasma Area and Severity Index (MASI) or others have used the mexameter.[32] We used a different grading scale in our studies, as the subjects also had postinflammatory hyperpigmentation from acne and as such cannot directly compare with previous results. Nevertheless, clearly, the PKEK-containing formulation was highly efficacious. Equally, it is difficult to compare between studies as differences in skin-lightening efficacy between different ethnic groups have been reported by Boissy *et al.*[18] Nevertheless, a 1 unit change in clinical efficacy was observed for both overall appearance and improvement in skin tone compared with vehicle in these skin types. Clearly, greater efficacy will be opened with additional skin-lightening actives, and as such, we have also demonstrated the additional activity of PKEK with sodium ascorbyl phosphate in Caucasian subjects over a 12-week period (unpublished results).

We have not attempted to understand the percutaneous absorption of PKEK but clearly the clinical results demonstrate that it is effective as a skin-lightening agent. Previous studies have demonstrated the effect of PKEK at the cellular level and have shown in skin biopsies reduced epidermal POMC, inflammatory cytokine and tyrosinase expression following UV irradiation.[34]

In conclusion, we have developed the tetrapeptide, PKEK, that has proven skin-lightening benefits on skin discoloration from melasma and postinflammatory skin hyperpigmentation. These studies have been conducted on subjects with skin types V-VI, but we believe this technology to be suitable for all skin types.

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