A randomized and placebo-controlled study to compare the skin-lightening efficacy and safety of lignin peroxidase cream vs. 2% hydroquinone cream

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Abstract

Keywords:
- skin lightening;
- lignin peroxidase;
- hydroquinone;
- LIP cream;
- skin whitening;
- skin brightening

Summary

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Aims The purpose of this study was to evaluate the skin-lightening efficacy and safety of lignin peroxidase (LIP) creams using a regimen of both day and night products compared with twice-daily application of 2% hydroquinone cream and placebo in Asian women.

Patients/Methods This was a randomized, double-blind, placebo-controlled, split-face, single-center study of 51 patients. Patients were randomized to receive day and night LIP cream on one randomly selected side of their face and either 2% hydroquinone cream or placebo on the other.

Results A statistically significant change from baseline in the melanin index was observed in LIP-treated skin, with a mean reduction of 7.6% ($P < 0.001$) on Day 31. Conversely, hydroquinone and placebo did not provide a statistically significant lightening effect when instrumentally measured. Dermatologist scoring demonstrated a significant improvement in overall fairness as early as 8 days after treatment initiation in the LIP-treated group, which was not observed in the other groups. Overall, patients preferred the LIP creams.

Conclusions The application of day/night LIP cream provided a significantly more rapid and observable skin-lightening effect than hydroquinone 2% cream or placebo.

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Introduction

Melanin, the dark pigment in the skin, is produced in the basal layer of the epidermis by melanocytes. It is stored in melanosomes, which are transferred to epidermal cells (keratinocytes) and transported to the upper layers of the epidermis to give the skin its typical color. Accumulation of melanin in the upper layers of the epidermis is the main cause for pigmentation disorders that are observed in all types of aging skin. Melanin is a very durable compound, and there is almost no available means of destroying its structure and lightening skin tone. Currently available treatments for skin lightening focus on the prevention of melanin formation by inhibiting its biosynthesis and/or preventing the stimulation of melanocytes by ultraviolet A (UVA) radiation or inflammation. Other methods include inhibiting the transfer of melanosomes from melanocytes to keratinocytes.

Historically, the most effective treatments for skin lightening have contained hydroquinone. However, safety concerns associated with hydroquinone have encouraged research into alternative agents to treat skin pigmentation disorders, including retinoids, mequinol, azelaic acid, arbutin, kojic acid, alesin, licorice extract, ascorbic acid, soy proteins, N-acetylglucosamine, and most recently, lignin peroxidase.

Lignin peroxidase is a naturally occurring enzyme derived from the tree fungus *Phanerochaete chrysosporium*. Lignin peroxidase has been identified as the enzyme that breaks down lignin in decaying trees, causing rapid decolorization. The molecular structure of lignin (an organic polymer found in the cell walls of plants) is similar to that of melanin, and recent research has confirmed that lignin peroxidase also has the potential to break down or depolymerize melanin. Lignin peroxidase is produced in a liquid form by Lonza of Switzerland in a proprietary, high-yield production process that produces a commercially concentrated lignin peroxidase (trademarked Melanozyme™ by Syneron, Yokneam Illit, Israel), which is formulated into a cosmetic product that can be used to improve the appearance of the skin for those who desire skin lightening and tone evenness (A. Khaiat,
unpublished data). Although the exact mechanism by which topically applied lignin peroxidase breaks down melanin in the epidermis is still being elucidated, in vitro studies have shown this effect to be contingent upon achieving an appropriate ratio of H$_2$O$_2$ and veratryl alcohol (VA) added to the lignin peroxidase. In many countries, H$_2$O$_2$ is allowed in concentrations up to 4% in cosmetic skin care products; the clinically irrelevant amount (0.012%) of H$_2$O$_2$ in the study product is instantly consumed, but is sufficient to potentiate the activity of lignin peroxidase. Research is currently being carried out to develop a new version of the product that does not contain H$_2$O$_2$. The presence of VA maintains the specific depolymerization process of melanin. Because the reaction is pH dependent (the activator is at pH 3.5, and the enzyme is only active at pH < 4.5), the return of the skin to its natural pH (i.e., around 5.5) will stop the reaction in <1 h.

The purpose of this study was to evaluate the skin-lightening efficacy and safety of lignin peroxidase (LIP) creams (using a regimen of both day and night products) compared with twice-daily application of commercially available 2% hydroquinone cream and placebo in Asian women.

**Methods**

This was a randomized, double-blind, controlled, paired, split-face, single-center study of 51 patients. Patients were randomized to receive day and night LIP cream on one randomly selected side of their face and either 2% hydroquinone cream or placebo on the other (ingredients listed in Appendix 1).

Included patients were women of Asian ethnicity between the ages of 20 and 60 years who agreed to either abstain from solar exposure or use UV protection for the duration of the study. The subject population was a mix of East Asian (Chinese and Korean) along with Southeast Asian (Filipino, Vietnamese, and Thai) ethnicity. Their Fitzpatrick skin types ranged from types 3–5. Patients were excluded if they had a pre-existing or dormant dermatologic condition (e.g. severe acne, atopic dermatitis, eczema, psoriasis, and skin cancer); had a history of a disease/condition or a concurrent illness that could interfere with the outcome of the study; or had used oral retinoids or steroids or used topical retinoids, alpha hydroxy acids, and/or beta hydroxy acids within the last month. Patients who had used products containing hydroquinone in the last 12 months or had undergone dermatologic treatments or procedures within the last month were also excluded. Other exclusion criteria included known sensitivity or allergy (as defined by the study investigator) to cosmetic ingredients, participation in a clinical investigation within the last 3 months, pregnancy, or lactation.

Study visits were at baseline and on Days 8, 15, and 31. Patients were dispensed their randomized, masked treatment regimens in four separate containers. Four of these products were masked lightening creams, with two creams labeled with instructions to apply in the morning (one for the left side and one for the right side of the face) and two were for evening. Patients were also dispensed an activator lotion for twice-daily use following the application of the creams. Products were labeled only as to patient identification number and instructions for use (frequency and which side of the face it was to be applied on). The participants were trained to apply a dime-sized cream to their face, twice daily for a period of 31 days. Patients were also provided with a cleanser for twice-daily use. Commercially available SPF 15 sunscreen was given to the participants, and it was recommended that they use it regularly during the study (especially if they spent any length of time in the sun).

The primary outcome measure was the evaluation of whole-face skin-lightening effect on the chin, cheeks, and forehead as measured by Mexameter® after 8, 15, and 31 days of twice-daily use of the assigned products.

Secondary outcome measures included the evaluation of the whitening effect as graded by a dermatologist using structured scales measuring overall fairness and mottled
hyperpigmentation, as well as visual evaluation of changes using the VisioFace® system and patients’ response to a subjective evaluation questionnaire.

Clinical measurements
Mexameter®
The Mexameter® used in this trial was a Courage + Khazaka (Köln, Germany) device and was equipped with a 5-mm-diameter head to measure the content of melanin and hemoglobin in the skin. The measurement derived from the Mexameter® is based on the absorption principle. The special probe of Mexameter® MX18 emits light of three predefined wavelengths (568, 660, and 870 nm). The melanin is measured by two of these wavelengths, chosen in order to achieve different absorption rates by the melanin pigments.

Measurements were taken from the forehead, the two cheeks, and the chin of each participant at baseline and on Days 8, 15, and 31. Two different wavelengths were used to measure the absorption capacity of the skin. One of these wavelengths corresponds to the spectral absorption peak of hemoglobin. The other wavelength has been chosen to avoid other color influences (e.g. bilirubin).

The primary outcome variable with the Mexameter® was the reduction in the melanin index. In addition, the masked investigator graded overall fairness on a scale of 0–9 (where 0 = perfectly fair and 9 = severely dark). Mottled hyperpigmentation was graded on a scale of 0–9 (where 0 = none and 9 = extremely blotchy). Mexameter® measures were taken in triplicate, and data are presented as the means of the three readings.

VisioFace®
The camera used in this study was a VisioFace® BW30 (Courage + Khazaka). At each study visit, the photographs were taken in standardized, white, and UV light after the patient placed her face to the front or to the side in a light facial booth. The diodes illuminate the face homogeneously. The camera and lights were both software-controlled and immediately ready for use.

Subjective questionnaire
On Days 8, 15, and 31, subjects completed a questionnaire regarding the efficacy of the product with regard to skin appearance, skin tone, radiance, and lightening/whitening, using a scale ranging from “agree completely” to “disagree completely”.

Statistical methods
Continuous data were analyzed using either paired-sample t-tests (for parametric variables) or Wilcoxon paired-sign rank tests (for nonparametric variables) as appropriate. The a priori level of statistical significance was \( \alpha = 0.05 \) for all tests. Excel and SAS (Version 9.1, SAS Institute, Cary, NC, USA) software were used for all analyses.

Results
Fifty-one patients enrolled and 41 completed the study. Two subjects withdrew their consent before starting the treatment. The mean age of participants was 38 years and 7 months. Of the 41 patients in the analysis, all 41 received LIP on one side of their face. On the other side of their face, 14 patients received 2% hydroquinone and nine received placebo (placebo included the same ingredient list as the LIP cream without the active ingredient, LIP).
Mexameter<sup>®</sup> readings

After 8 days of treatment, there was a statistically significant change from baseline in the melanin index in LIP-treated skin, with a mean reduction of 4% among the 71% of patients who responded to treatment. This trend continued to a 7.6% mean reduction in melanin index after treatment ($P < 0.001$) among the 83% of patients who responded at 31 days. Conversely, the changes in the 2% hydroquinone group and the placebo group did not achieve statistical significance during the study (Fig. 1). Similarly, skin treated with LIP was more likely to exhibit an improvement in melanin index than skin treated with hydroquinone (Table 1 and Fig. 2).
The change in skin color, which was observed by the Mexameter® readings when compared with baseline, was clearly seen in the VisioFace® photographs. Photographs 1 and 2 show the Subjects 2 and 19 following the use of LIP cream and 2% hydroquinone, respectively, twice daily for 31 days.

Table 1. Mexameter® results for LIP, hydroquinone, and placebo after 31 days

<table>
<thead>
<tr>
<th>Mexameter® parameters</th>
<th>Kinetic</th>
<th>Δ (mean ± SEM)</th>
<th>Δ% on average</th>
<th>Student's t-Test</th>
<th>Significant</th>
<th>Subjects with positive effect (%)</th>
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<tbody>
<tr>
<td>LIP cream</td>
<td>Δ D8</td>
<td>-9.55 ± 2.85</td>
<td>-3.76</td>
<td>0.003</td>
<td>Yes</td>
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</tr>
<tr>
<td></td>
<td>Δ D31</td>
<td>-18.18 ± 3.70</td>
<td>-7.56</td>
<td>&lt;0.001</td>
<td>Yes</td>
<td>82.93</td>
</tr>
<tr>
<td>2% hydroquinone</td>
<td>Δ D8</td>
<td>2.36 ± 4.47</td>
<td>0.89</td>
<td>0.304</td>
<td>No</td>
<td>35.72</td>
</tr>
<tr>
<td></td>
<td>Δ D31</td>
<td>4.02 ± 4.77</td>
<td>1.50</td>
<td>0.227</td>
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<tr>
<td>Placebo</td>
<td>Δ D8</td>
<td>2.31 ± 3.69</td>
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<td>0.274</td>
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<tr>
<td></td>
<td>Δ D31</td>
<td>-0.52 ± 4.16</td>
<td>-0.26</td>
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<td>44.44</td>
</tr>
</tbody>
</table>

LIP, Lignin peroxidase.

Figure 2. Percent of patients exhibiting improvement in melanin index compared with baseline. Improvement was defined as a significant decrease in the melanin index.
Figure Photograph 1. (a) Subject 2 at baseline (time 0). (b) Subject 2 after 31 days of treatment with LIP cream.
When considering the comparison of LIP-treated skin with the other products applied to the opposite side of the same patient’s face, the mean changes from baseline were consistently greater with LIP (range: −15.1 to −19.2) than with hydroquinone (mean: −4.0 ± 4.8) and placebo (−0.5 ± 4.2). However, a statistically significant difference between LIP and hydroquinone was observed only after 31 days but not after 8 days. In the case of LIP vs. placebo, the difference was significant only at Day 31. This small deviation might be related to the smaller sample sizes for these subanalyses.

The LIP regimen provided a faster and greater improvement than the other treatments in overall fairness scores, with a mean decrease of −1.9 ± 0.1 (P < 0.001). A decrease in score represents an improvement in fairness. Improvements in the percent reduction of scores from baseline were noted with the LIP and hydroquinone, but not with the placebo group (Fig. 3). Similarly, the LIP regimen provided the greatest improvement in mottled hyperpigmentation scores compared with the other treatments, with a mean decrease of −1.9 ± 0.2 (P < 0.001). Improvements in the percent reduction of scores from baseline were noted with the two treatments, but not with the placebo group (Fig. 4).
Subjective questionnaire

Overall, patients responded that the LIP creams were preferred, with more patients selecting the regimen for making skin look healthier, more radiant, fairer/lighter, more even-toned, smooth and refined, and translucent (Fig. 5).

Adverse events

Eight patients experienced mild adverse events (AEs) and withdrew from the study. The five patients who reported mild AEs on the LIP side of their face also reported the same AEs on the control side of their face; therefore, it was concluded that these AEs were not related to the active ingredient of LIP treatment.

Erythema was not observed by the dermatologist, nor was it measured by Mexameter® in the LIP-treated group.

Discussion

The findings of the present study suggest that a LIP skin-lightening treatment system is an effective regimen in Asian women. Objective instrumental measurements taken with the Mexameter® demonstrated that the LIP day/night regimen provided a statistically significant skin-lightening effect, which was observed much earlier than the effects of the other treatments and reached a more satisfactory degree of lightening. Conversely, skin treated with 2% hydroquinone cream or placebo failed to provide statistically significant improvement.

In the subjective patient evaluation, more patients reported a significant improvement in overall fairness, overall smoothness, healthiness, and evenness of tone with the LIP regimen than with the other products.

There are limitations to the current study owing to the relatively small sample size (n = 41 evaluable subjects) and the inclusion of only one racial/ethnic group, Asian skin (FST III-V). However, this product has been tested in other human studies and showed a similar safety profile and same early observable lightening effect.  

The short duration of study follow-up was chosen mainly to show the rapidity of effect. Skin-lightening products often are expected to be used for more than 30 days in order to attain full
effectiveness. The LIP skin-lightening treatment system was designed to eliminate/reduce the superficial melanin, not to go deep into the skin. It is the superficial melanin that is visible from the outside and that layer is reduced with the treatment. The LIP does not interfere with the mechanism of melanogenesis, and therefore, its effect is seen much earlier than other products, which are directed to prevent melanin synthesis and need to be used for a longer time before the effect on the skin surface can be observed.

This randomized, double-blind, placebo-controlled clinical trial was designed purposely to compare the effectiveness of a LIP-based regimen with that of other commercially available products. In the future, more human clinical trials will be performed to demonstrate the efficacy of the LIP technology in the treatment for common skin pigmentation disorders such as melasma and age spots.

Conclusion
The application of day/night LIP cream provided statistically significant skin lightening, while the other products did not. More patients reported satisfactory results with the LIP regimen than with 2% hydroquinone cream and placebo. Longer-term studies are needed to confirm these findings. A comparative study of twice-daily use of LIP cream vs. 4% hydroquinone cream for skin lightening is currently under way.

References
AbstractFull Article (HTML)PDF(420K)References


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AbstractFull Article (HTML)PDF(11909K)


Appendix
Appendix 1. List of ingredients in formulations used in this study

Lignin peroxidase day cream: purified water, glycerin, trehalose, pullulan, heptyl undecylenate, cetyl palmitate, myristyl myristate, dicaprylyl carbonate, caprylic capric triglyceride, tribhehenin, jojoba ester, cetyl alcohol, stearyl alcohol, polyisobutene, polysorbate 20, polyacrylate 13, phenoxyethanol, 3,4-dimethoxybenzyl alcohol, ligninase, hydrogen peroxide, citric acid.

Lignin peroxidase night cream: purified water, glycerin, trehalose, pullulan, propylene glycol, ethoxydiglycol, dimethicone, cetyl palmitate, myristyl myristate, dicaprylyl carbonate, caprylic capric triglyceride, Butyrospermum parkii, jojoba ester, cetyl alcohol, stearyl alcohol, polyisobutene, polysorbate 20, polyacrylate 13, phenoxyethanol, 3,4-dimethoxybenzyl alcohol, ligninase, hydrogen peroxide, citric acid.

Two percent hydroquinone cream: water, mineral oil, glyceryl stearate, cetyl alcohol, isopropyl palmitate, PEG-100 stearate, propylene glycol, emulsifying wax NF, stearic acid, hydroxyethylcellulose, tocopheryl, acetate, magnesium ascorbyl phosphate, beta-carotene, disodium EDTA, C13-14 isoparaffin, sodium meta-bisulfite, citric acid, BHT, methylparaben, propylparaben, diazolidinyl urea, fragrance, hydroquinone USP—2% skin lightener, octinoxate—2.5% sunscreen.