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Clinical Efficacy of 25% L-Ascorbic Acid (C'ensil) in the Treatment of Melasma

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Background: L-Ascorbic acid is used to treat melasma; however, it is quickly oxidized in aqueous solutions. Thus, C'ensil, a formulation containing 25% L-ascorbic acid and a chemical penetration enhancer, was created to promote the penetration of L-ascorbic acid into the skin.

Objective: To evaluate the efficacy of C'ensil in patients with melasma.

Methods: Forty subjects with melasma were treated with C'ensil during an open-label trial over a period of 16 weeks. Each subject's skin pigmentation was assessed every 4 weeks using the Melasma Area and Severity Index (MASI) and mexameter score. In addition, transepidermal water loss, skin dryness and irritation, and quality of life (Melasma Quality of Life Scale (MelasQoL)) were evaluated.

Results: After 16 weeks, a significant decrease was noted in the degree of pigmentation based on the patients' MASI and mexameter scores. MelasQoL scores also decreased, indicating an increase in the subjects' quality of life.

Conclusion: Our data indicate that C'ensil is an effective treatment modality for melasma.

Antécédents: L'acide ascorbique est utilisé dans le traitement du mélasma. Toutefois, cet acide subit une oxydation rapide dans les solutions aqueuses. Le recours au C'ensil, une préparation à base de 25 % d'acide ascorbique et d'un activateur de pénétration chimique, a été mis au point dans la perspective d'améliorer l'absorption de l'acide ascorbique par la peau.

Objectif: Évaluer l'efficacité de C'ensil chez les patients souffrant de mélasma.

Méthodes: Quarante patients atteints de mélasma ont été traités au C'ensil pendant 16 semaines dans le cadre d'un essai ouvert. La pigmentation cutanée de chaque sujet a été évaluée aux 4 semaines au moyen de l'index mélanique (mexameter) et du score clinique (MASI). Par ailleurs, ont a procédé à une évaluation de la perte d'eau transépidermique, du niveau de sécheresse,et d'irritation de la peau ainsi que la qualité de vie (MelasQoL).

Résultats: Aprés 16 semaines, une nette baisse dans la pigmentation a été remarquée selon les scores cliniques et l'index mélanique. Les scores MelasQoL ont baissé, indiquant une amélioration à la qualité de vie des sujets.

Conclusion: Nos données montrent que C'ensil est un véhicule de traitement efficace du mélasma.

MELASMA, also referred to as common acquired hypermelanosis, is characterized by irregular brownish macules and patches involving the sun-exposed areas of the skin. Several therapeulic modalities have been used to treat melasma, including chemical peels, laser therapies, and topical agents such as azelaic acid, hydroquinone, kojic acid, and retinoic acid[1-3] ; however, there is no gold standard treatment for melasma.

Ascorbic acid, which suppresses melanin synthesis via an antioxidant effect and/or tyrosinase inhibition, has been successfully used to treat photodamage[4-7] ; however, it is quickly oxidized in aqueous solutions. To overcome this limitation, modified treatments using L-ascorbic acid have been introduced, including magnesium L-ascorbyl-2-phosphate (MAP) vitamin C iontophoresis.[8-10]

Here we present a clinical evaluation of C'ensil (Sage Pharmaceuticals lnc., Shreveport, LA), a new treatment system for melasma that contains 25% L-ascorbic acid and a chemical penetration enhancer consisting of N-methyl-2-pyrrolidone and dimethyl isosorbide.

Material and Methods

Patients and Methods

Forty women with mild to severe melasma were enrolled in the study. Subjects were between 26 and 52 years of age, in

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good general health, and had a Fitzpatrick skin color classification of type III or IV. Our exclusion criteria included the use of tanning parlors; pregnancy; the use of hydroquinone within 3 months prior to the study; treatment with topical or systemic analogues of vitamin A, including tretinoin, within the previous 3 months; chemical abrasion, microdermabrasion, or laser treatment within 9 months prior to the study; and the use of topical steroids on the face within 1 month prior to the study. In addition, patients using oral contraceptives or hormone replacement therapy were excluded from the study.

All 40 subjects were treated as part of an open-label study with 25% L-ascorbic acid and a chemical penetration enhancer consisting of N-methyl-2-pyrrolidone and dimethyl isosorbide (C'ensil) for 16 weeks. Twice daily, the patient applied the study serum to their entire face. The application of other topical agents used to treat melasma was not permitted, except for sunscreen. Following the initial evaluation, each patient was evaluated every 4 weeks during the entire 16-week period. Digital photographs (frontal, right, and left views) were taken during each evaluation with a Canon 300D digital camera (Canon Inc., Tokyo, Japan).

Clinical Assessments of Efficacy

The degree of pigmentation was assessed at each visit using the minimum Melasma Area and Severity Index (MASI) and the mexameter score. [11] In addition, a tewameter was used to measure transepidermal water loss (TEWL), and a sebumeter was used to assess skin dryness and irritation.

The MASl is calculated on the basis of the area involved, the shade of the melasma, and the homogeneity of hyperpigmentation. Four areas of the face were evaluated ; forehead(f), right malar(rm), left malar(lm), and chin(c), which represent 30%, 30%, 30%, and 10% of the facial surface, respectively. The area (A) of involvement in each of the four regions was assigned a numeric value between 0 and 6 as follows: 0, no involvement; 1, 1 to 9%; 2,10 to 29%; 3, 30 to 49%; 4, 50 to 69%; 5, 70 to 89%; and 6,90 to 100%. The severity of melasma was based on two factors, darkness (D) and homogeneity (H), which were measured on a scale of 0 to 4 as follows: 0, absent; 1, slight; 2, mild; 3, marked; and 4, maximum. The equation used to determine the facial MASI was 0.3[D(f) + H(f)A(f)) + O.3[D(rm) + H(rm)A(rm)) + O.3[D(lm) + H(lm)A(lm)] + O.1[D(c) +H(c)A(c)]; the maximum possible score was 48, whereas the minimum possible score was 0.

The mexameter (Courage & Khazaka Electronics, Cologne, Germany) provided accurate (±5%) and reproducible estimates of melanin content. The values on a mexameter range from 1 to 1,000 (0 = white and 1,000 = black). We assigned mexameter scores for three to five hyperpigmented macules within each melasma area. To evaluate the whitening effect of the treatment serum on the melasma-uninvolved areas, additional readings were taken from five melasma-uninvolved area (glabella, center of the forehead, both zygomatic prominences, and chin). To control for changes in pigmentation owing to tanning, a mexameter score was assigned for each patient's forearm since that area was exposed to light but not to the study serum.

A TM-300 probe (Courage & Khazaka Electronics) was used to measure TEWL at two sites (the junction between the left midsagittal browline and the horizontal line of the forehead and the left zygomatic prominence), and a sebumeter (Courage & Khazaka Electronics) was used to determine the amount of sebum on the skin surface at two sites (the junction of the right midsagittal browline and the horizontal line of the forehead and at the right zygomatic prominence).

The MASI, mexameter, tewameter, and sebumeter data, which were collected at weeks 4, 8, 12, and 16, were analyzed using repeated measures.

The effects of the melasma on each patient's quality of life were assessed using the Melasma Quality of Life Scale (MelasQoL)[12] at weeks 0 and 16 (Table 1).

Clinical Assessment of Safety and Tolerability

Side effects such as stinging, burning, and pruritus were recorded by the patients. Also, investigators evaluated the patients for the presence of erythema, dryness, scaling, desquamation, and postinflammatory hyperpigmentation.

Table 1. Ten Questions of the Melasma Quality of Life Scale

On a scale of 1 (not bothered at all) to 7 (bothered all the time), the patient rates the following ;

1. The appearance of the skin condition
2. Frustration about the skin condition
3. Embarrassment about the skin condition
4. Feeling depressed about the skin condition
5. The effects of the skin condition on interaction with other people (eg, interaction with family, friends, close relationships) .
6. The effects of the skin condition on desire to be with people
7. Skin condition making it hard to show affection
8. Skin discoloration making the patient feel unattractive to others
9. Skin discoloration making the patient feel less vital or productive
10. Skin discoloration affecting the patient's sense of freedom

This study was approved by the Inje University Institutional Review Board, and all patients gave informed written consent. The subjects were recruited from the Department of Dermatology, Inje University of College of Medicine, Busan, Korea.

Statistical Analysis

Statistical analysis of the data (MASI, TEWL, mexameter, and sebumeter) from each visit was carried out by repeated analysis of variance. A p value < .005 was considered to be statistically significant.

Results

Thirty-nine of 40 subjects completed the entire 16-week trial. The MASI, mexameter, sebumeter, and tewameter data, which were collected in person and by the MelasQoL, are summarized in Table 2.

Based on a comparison of the photographs taken at baseline and after 16 weeks, a steady improvement in the degree of melasma was detected (Figure 1).

Efficacy Analysis

MASI values significantly decreased from baseline to week 16 (from 15.60 to 12.03, P < .0001; Figure 2). This indicates that the study serum was effective in the treatment of melasma.

Our mexameter results indicate a significant decrease in the degree of pigmentation at the treated sites between weeks 0 and 16 (from 215.01 to 198.75, P < .0001; Figure 3A), whereas a slight increase in the forearm mexameter score was noted. The mexameter scores of the melasma-uninvolved areas were also significantly decreased between weeks 0 and 16 (from 189.95 to 182.75, P < .0001; Figure 3B). This implies that the study serum had a whitening effect on all areas, not Just those affected by melasma; however, the mexameter readings taken at the forearm showed no significant change throughout the course of the study.

The tewameter scores showed a slight increase from baseline to week 4; however, they gradually decreased from week 4 to week 16 (Figure 4A). The sebumeter scores also decreased from baseline to week 8, but they increased from week 8 to week 16 (Figure 4B). This indicates that the side effects of the serum, including impaired barrier function, irritation, and dryness, were temporary and gradually decreased.

The MelasQoL scores decreased from 39.85 to 36.23 over the course of the study, indicating that the treatment serum improved the patients' self-reported quality of life (Figure 5).

Safety Assessments

During the study periods, 30 patients experienced stinging, 23 experienced burning, and 6 experienced pruritus. No patient had severe adverse effects, and all patients were able to continue the study. Eight patients showed erythema, and five patients showed scaling. However, these side effects were mild and temporal. Only three patients showed mild to moderate stinging, and two showed mild to moderate burning. However, these side effects disappeared within 2 weeks.

Postinflammatory hyperpigmentation was not observed during the study period for all enrolled patients.

Discussion

Melasma, or common acquired symmetric hypermelanosis, is characterized by irregular brownish macules and patches involving the sun-exposed areas of the skin. The etiologic factors associated with melasma include genetics, ultraviolet radiation (UVR), nutritional deficiencies,

Table 2. Overall Results of the MASl; Mexameter Score Measured at Melasma Lesions, Melasma-Uninvolved Areas, and Forearms; Sebumeter Score; TEWL; and MelasQoL

Treatment Period	Baseline	4 wk	8 wk	12 wk	16 wk
MASI	15.60	14.30	13.25	12.82	12.03
Mexameter (melasma)	215.01	207.81	209.28	204.84	198.75
Mexameter (melasma-uninvolved area)	189.95	189.43	188.53	187.22	182.75
Mexameter (forearm)	140.25	142.08	144.05	143.62	143.33
Sebumeter	13.26	9.11	8.81	10.27	10.17
Tewameter	9.15	10.90	10.30	9.91	9.88
MelasQoL	39.85				36.23

MASI = Melasma Area and Severity Index ; MelasQoL = Melasma Quality of Life Scale; TEWL = transepidermal water loss

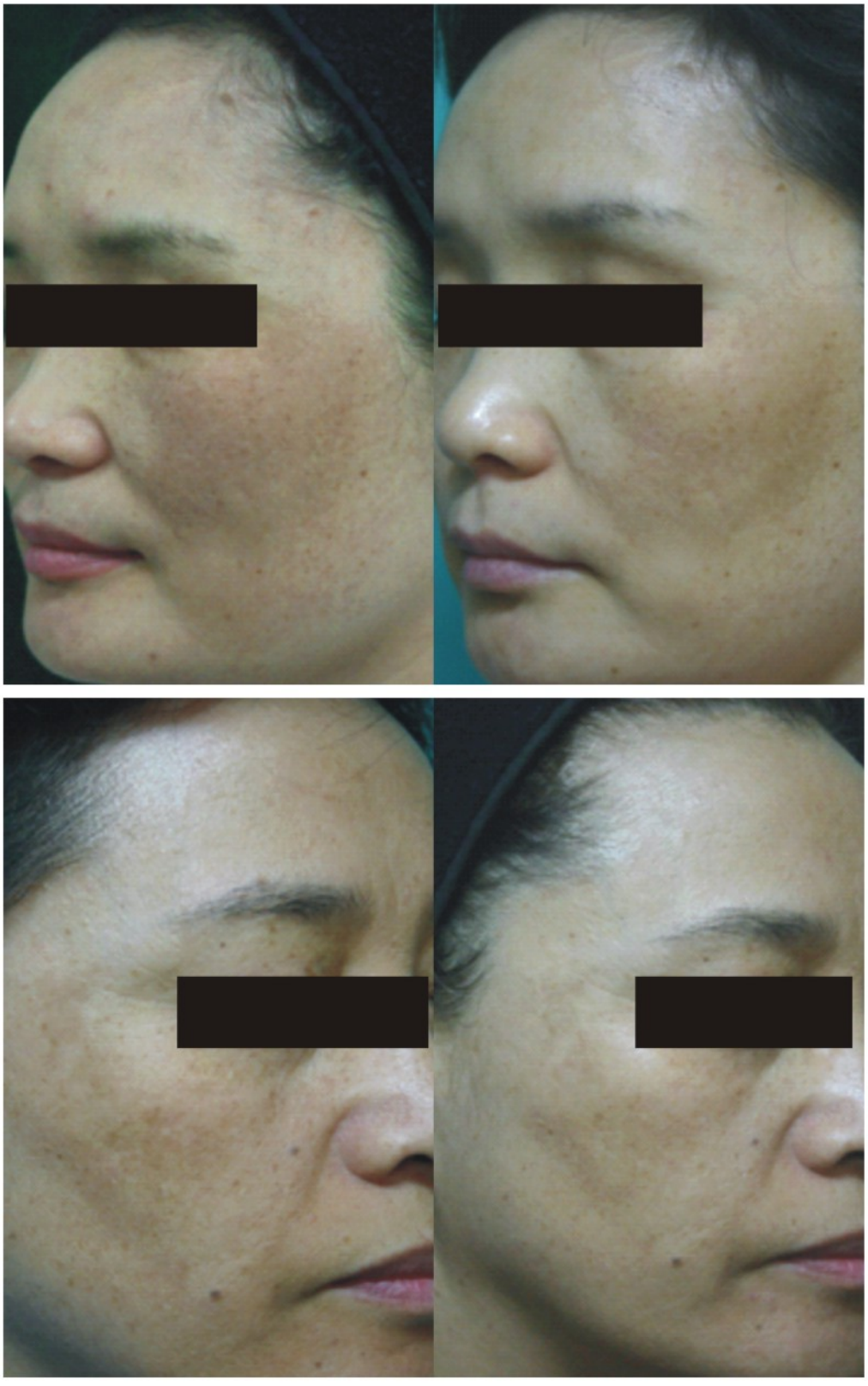

Figure 1. Representative cases showing marked lightening of melasma at 16 weeks.

medications, hormonal influences owing to pregnancy, and oral contraceptives[2]; however, of these, UVR is the most commonly cited.

UVR may directly stimulate melanogenesis by striking the lipids in the plasma membrane of melanocytes, resulting in the release of diacylglycerol into the cytoplasm and the activation of tyrosinase.[13] With regard to its indirect effects on melanocytes, UVR induces keratinocytes to synthesize several paracrine melanocyte factors (eg, basic fibroblast growth factor, endothelin-1, α-melanocyte-stimulating hormone (MSH), and prostaglandin E_2 [PGE_2]) that stimulate melanocyte proliferation. UVR

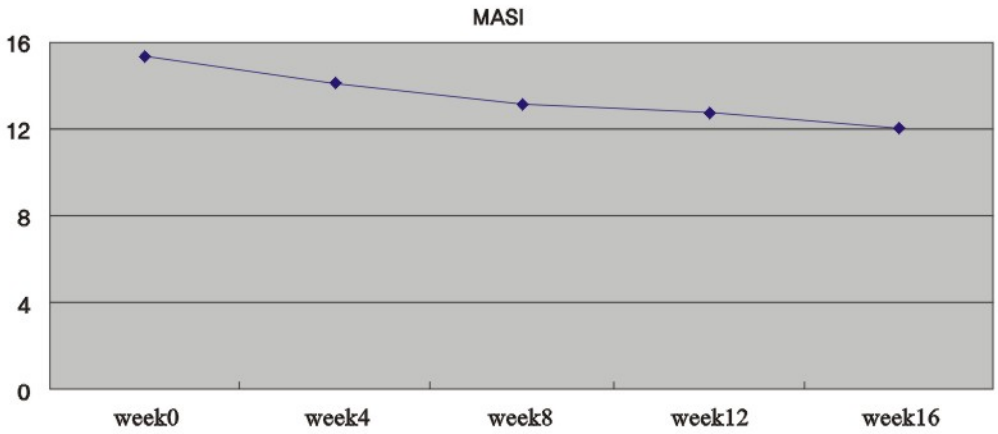

Figure 2. There was a statistically significant decrease in the Melasma Area and Severity Index from baseline to week 16 ($p < .0001$).

also induces the formation of reactive oxygen species (ROS), which initiate oxidation reactions during melanogenesis, assist in melanin biosynthesis, and induce melanocyte proliferation.[14-16]

Ascorbic acid inhibits melanin production by diminishing the ability of UVR to stimulate the synthesis of melanin.[5-7] Presumably, it does so by acting as a nonenzymatic antioxidant to neutralize free radicals generated

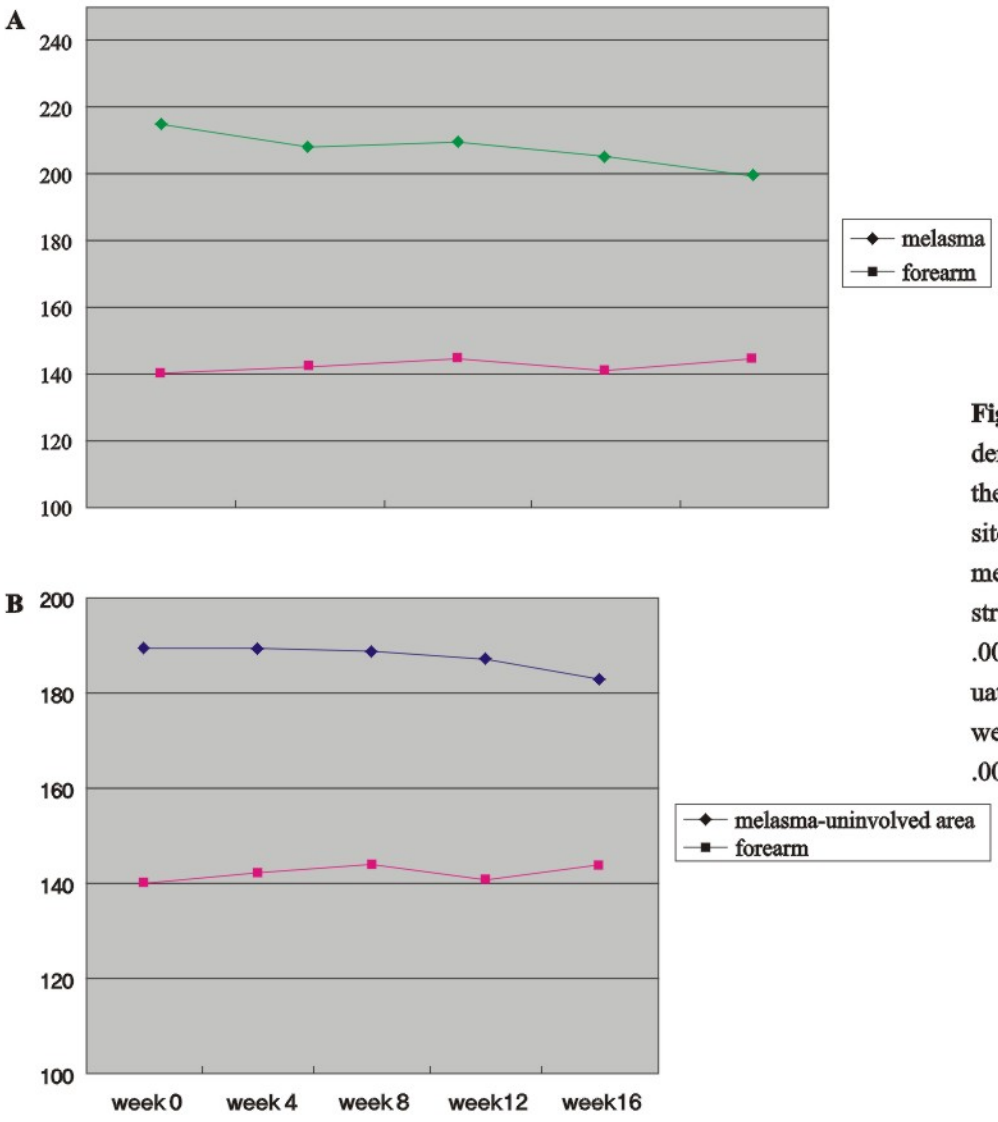

Figure 3. A, The mexameter results demonstrated a significant decrease in the degree of pigmentation at treated sites, whereas the mexameter score measured at the forearm demonstrated no significant change ($p < .0001$). B, The melasma scores evaluated at melasma-uninvolved areas were significantly decreased ($p < .0001$).

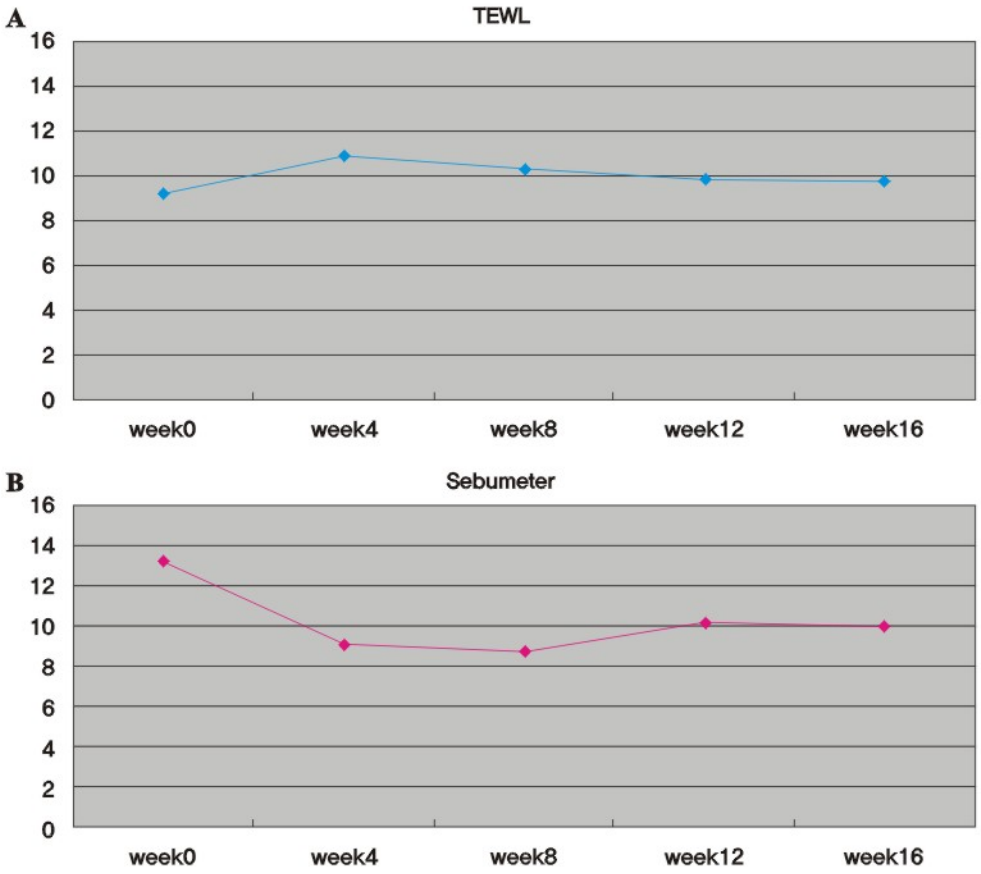

Figure 4. A, The tewameter score showed a slight increase from baseline to week 4. But the score gradually decreased from 4 to 16 weeks. B, The sebumeter score decreased from baseline to week 8 but showed an increase from 8 to 16 weeks. TEWL= transepidermal water loss

in the aqueous compartment of the cell. In addition, ascorbic acid suppresses the induction of melanocyte proliferation factors such as interleukin-1α, α-MSH, and PGE_2 by ROS.

In the melanin synthetic pathway, tyrosinase is a key enzyme in the synthesis of melanin within the melanocyte by catalyzing the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and oxidation of

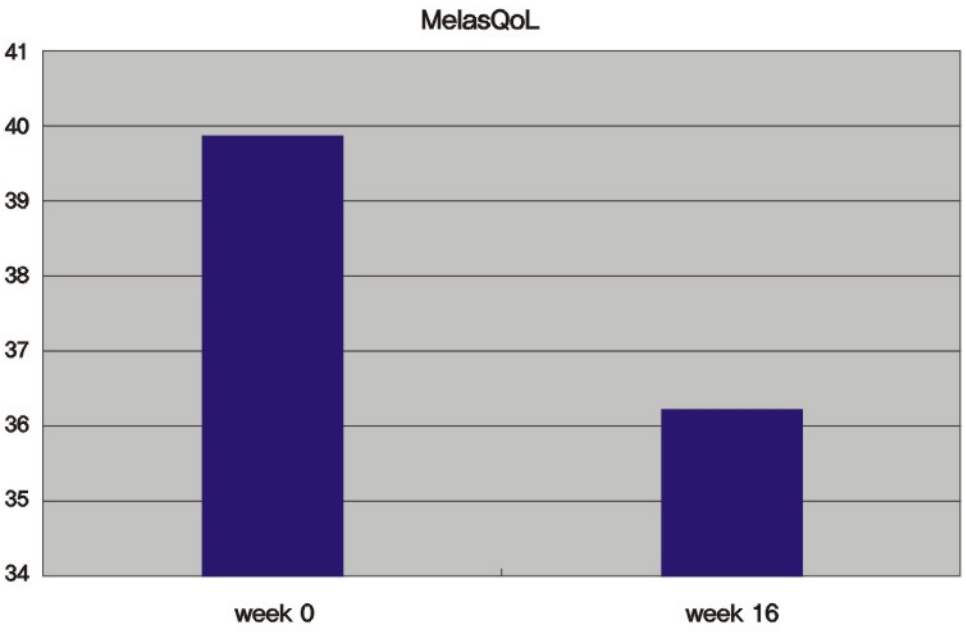

Figure 5. The Melasma Quality of Life Scale (MelasQoL) decreased from baseline to 16 weeks, which revealed that the treatment using the study cream increased the patients' subjective attractiveness and satisfaction.

DOPA to dopaquinone. Also, ascorbis acid exerts its depigmenting effect by inhibiting the peroxidase-catalyzed melanogenic reactions in melanocytes.[13-15] Consequently, ascorbic acid may change the pigmentation from black to tan and then show an important effect on melasma. However, ascorbic acid is water soluble; thus, the lipid-rich stratum corneum of the epidermis is highly resistant to penetration by ascorbic acid-containing solutions.[8-10] Also, ascorbic acid is quickly oxidized in aqueous solutions and thus is not generally useful as a depigmenting agent. To solve these problems, Pinnell and colleagues suggested that L-ascorbic acid be formulated at a pH less than 3.5.[17] According to their data, the optimal concentration for maximal percutaneous absorption was 20%; however, the formulation they used induced irritation owing to its acidity. Ascorbic acid derivatives such as MAP and ascorbic-6-palmitate are stable in aqueous solutions; thus, they are capable of greater skin penetration. However, following penetration, they remain on the extracellular surface in derivative form, meaning that less of the compound is converted into L-ascorbate.[8-10] To enhance ascorbic acid penetration through skin, iontophoresis has been used in several studies and was shown to have a satisfactory effect on melasma.[10,11] Iontophoresis increases the penetration of drugs or other compounds into the tissue as a result of an applied current through the tissue. Huh and colleagues used iontophoresis to enhance MAP penetration through the skin of 29 patients with melasma[10] In their study, iontophoresis with MAP showed effective depigmenting effects. However, the procedure is difficult to perform and cannot be performed on individuals with pacemakers or pregnant women.

The cosmetic formula used in this study, C'nsil, contains 25% L-ascorbic acid and a combination of N-methyl-2-pyrrolidone and dimethyl isosorbide. N-methyl-2-pyrrolidone is a good solvent for water-soluble drugs and increase the transdermal absorption of compounds by enhancing penetration. Modifying the penetration of ascorbic acid appears to stabilize it in aqueous solution.[18,19] In addition, dimethyl isosorbide stimulates drug penetration by reducing the water content of the stratum corneum. [19,20] Thus, the formulation used in this study can elevate the percutaneous concentration of L-ascorbic acid. L-ascorbic acid is known to inhibit melanin formation by reducing o-quinones and reduce oxidized melanin, so melanin cannot be formed by the action of tyrosinase until L-ascorbic acid is oxidized. In these respects, C'ensil, which contains 25% L-ascorbic acid and penetration enhancers, can show depigmenting effects by increasing percutaneous penetration of L-ascorbic acid and by using highly concentrated L-ascorbic acid.

Our results show that C'ensil is an effective therapeutic agent for the treatment of melasma. Our assessment was based on objective methods, such as mexameter readings and MASI values. In addition to the demonstrated efficacy of treatment, the estimated mexameter scores for the melasma-uninvolved areas also showed statistically significant improvement, implying that the study serum had general whitening effects.

The tewameter and sebumeter scores indicated a deterioration of the skin barrier early on in the study. The acidic characteristics of the study serum and the reduction in water content in the stratum corneum by the chemical enhancer contained in the serum can induce skin irritation and barrier impairment ; however, these seemed to be temporary effects because the tewameter and sebumeter scores gradually improved.

Moreover, our MelasQoL results revealed a significant improvement by the end of the study. This implies that the study serum improved the patients' sense of attractiveness and satisfaction.

Conclusion

A new cosmetic formula system, C'ensil, which contains 25% L-ascorbic acid and uses the chemical penetration-enhancing combination of N-methyl-2-pyrrolidone and dimethyl isosorbide, is effective not only for the treatment of melasma but also for lightening of the skin. The possible side effects of this formulation are temporary and tolerable.

References

1. Khalifa ES, Mohammad MA, Sabeeh AA. Lactic acid as a new therapeutic peeling agent in melasma. Dermatol Surg 2005;31;149-54
2. Ian LG, Amit GP. Safety and efficacy of 4% hydroquinone combined with 10% glycolic acid, antioxidants, and sunscreen in treatment of melasma. Int J Dermatol 2003;42;966-72
3. Hermannes F, Petit L, Pierard-Franchimont C, et al. Assessment of topical hypopigmenting agents on solar lentigines of Asian women. Dermatology 2002;204;281-6
4. Behrooz K. Peroxidase-mediated mechanisms are involved in the malnocytotoxic and melanogenesis-inhibiting effects of chemical agents. Dermatology 2002;205;329-39
5. Smit N, Vicanova J, Cramers P, et al. The combined effects of extracts containing carotenoids and vitamins E and C on growth and pigmentation of cultured human melanocytes. Skin Pharmacol Physiol 2004;17:238-45
6. Raschke T, Koop U, Filbry A, et al. Topical activity of ascorbic acid; from in vitro optimization in vivo efficacy. Skin Pharmacol Physiol 2004;17;200-6

7. Seite S, Bredoux C, Zucchi H, et al. Histological evaluation of a topically applied retinol-vitamin C combination. Skin Pharmacol Physiol 2005;18:81-7
8. Kameyama K, Sakai C, Kondoh S, et al. Inhibitory effect of magnesium L-ascorbyl-2 phosphate (VC-PMG) on melanogenesis in vitro and in vivo. J Am Acad Dermatol 1996;34;29-33
9. Parl YK, Chung WS, Lee H, Jung SW. Whitening effects of cosmetics containing magnesium L-ascorbyl-2-phosphate (VC-PMG, vitamin C dermivatives) assessed by colorimeter. Ann Dermatol 2002;14;63-70.
10. Huh CH, Seo KI, Park JY, et al. A randomized, double-blind, placebo-controlled trial of vitamin C iontophoresis in melasma. Dermatology 2003;206;316-20
11. Yoo JM, Park HJ, Choi SW, Kim HO. Vitamin C-iontophoresis in melasma. Korean J Dermatol 2001;39;285-91
12. Kimbrough-Green CK, Griffiths CM, Finkel LJ. Topical retinoic acid (tretinoin) for melasma in black patients. Arch Dermatol 1994;130;727-33.
13. Balkrishnan R, McMichael AJ, Camacho FT, et al. Development and validation of a health-related quality of life instrument for women with melasma. Br J Dermatol 2003;149;572-7.
14. Gilchrest BA, Eller MS. DNA photodamage stimulates melanogenesis and other photoprotective responses. J Investing Dermatol Symp Proc 1999;4:35-40
15. Kojima S, Yamaguchi H, Morita K, Ueno Y. Inhibitory effect of sodium 5,6-benzylidene ascorbate(SBA) on the elevation of melanin biosynthesis induced by ultraviolet-A (UV-A) light in cultured B-16 melanoma cells. Biol Pharm Bull 1995;18:1076-80.
16. Darr D, Combs S, Dunston S, et al. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. Br J Dermatol 1992;127:247-53.
17. Pinnell SR, Yang H, Omar M, et al. Topical L-ascorbic acid: percutaneous absorption studies. Dermatology 2001;27:137-42.
18. Koizumi A, Fujii M, Kondoh M, Watanabe Y. Effect of N-methyl-2-pyrrolidone on skin permeation of estradiol. Eur J Pharm Biopharm 2004;57:473-8.
19. Kanikkannan N, Kandimalla K, Lamba SS, Singh M. Structure-acitivity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 1999;6:593-608.
20. Squillante E, Needham T, Maniar A, et al. Codiffusion of propylene glycol and dimethyl isosorbide in hairless mouse skin. Eur J Pharm Biopharm 1998;265-71.