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Effects of Trans-4-(Aminomethyl) Cyclohexanecarboxylic Acid/Potassium Azeloyl Diglycinate/Niacinamide Topical Emulsion in Thai Adults With Melasma: A Single-Center, Randomized, Double-Blind, Controlled Study

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ABSTRACT

BACKGROUND: Melasma is an acquired hyperpigmentary disorder characterized by dark patches or macules located on the cheeks, forehead, upper lip, chin, and neck. Treatment of melasma involves the use of topical hypopigmenting agents such as hydroquinone, tretinoin, and azelaic acid and its derivatives.

OBJECTIVE: The purpose of this study was to assess the efficacy of a formulation containing a combination of trans-4-(aminomethyl) cyclohexanecarboxylic acid/potassium azeloyl diglycinate/niacinamide compared with an emulsion-based control in the treatment of melasma in Thai adults.

METHODS: In this single-center, randomized, double-blind, controlled study, Thai patients with mild to moderate facial melasma (relative melanin value [RMV] in range of 20–120) were randomized for the application of either the test or the emulsion-based (control) product in the morning and before bedtime for 8 weeks. The supplemental sunscreen product with sun protection factor 30 was distributed to all patients. Subjects were assessed for the intensity of their hyperpigmented skin area by measuring the difference in the absolute melanin value between hyperpigmented skin and normal skin (RMV). This parameter was used as a primary outcome of this study. Additionally, the severity of melasma was determined visually using the Melasma Area and Severity Index (MASI) scored independently by 3 investigators. The assessments of melasma intensity and other skin properties were performed before administration (week 0) and every 2 weeks thereafter for up to 8 weeks. Other skin properties, including moisture content, pH, and redness (erythema value), were measured. Adverse events (AEs), including erythema, scaling, and edema, were also assessed by a dermatologist using the visual grading scale of Frosch and Kligman and COLIPA.

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RESULTS: The resulting primary intent-to-treat (ITT) population included 33 patients in the test group and 34 patients in the control group. Sixty patients completed all 8 weeks of the study (on-treatment [OT] population): 91% (30) of the 33 patients in the test group, and 88% (30) of the 34 patients in the control group. Between-group differences in mean RMV were statistically significant at week 6 in both the primary ITT ($P = 0.005$) and OT ($P = 0.006$) populations. The significant differences in mean MASI scores between the test and the control groups were initially observed at weeks 4 ($P = 0.005$) and 8 ($P = 0.027$) in the OT and primary ITT populations, respectively. Other parameters, including skin pH, erythema, and moisture content did not significantly change from baseline at any time point of study. The incidence of AEs was not different between the test (4/33 [12%]) and control (5/34 [15%]) groups.

CONCLUSIONS: The significant differences in RMVs between the test and control groups were observed after 6 weeks of treatment, both in the primary ITT and OT populations. The incidence of patients with AEs was not significantly different between the test and control groups. (*Curr Ther Res Clin Exp.* 2010;71:345–359)

KEY WORDS: trans-4-(aminomethyl) cyclohexanecarboxylic acid/potassium azeloyl diglycinate/niacinamide, melasma, Thai adults.

INTRODUCTION

Melasma is an acquired hyperpigmentary disorder characterized by dark patches or macules located on the cheeks, forehead, upper lip, chin, and neck.[1,2] This disorder is predominantly found in females, accounting for ~90% of all cases. It appears in all racial types, but occurs more frequently in persons with darker complexions (Fitzpatrick's skin type IV through VI).[3,4] The prevalence of melasma in Latino females varies from 1.5% to 33.3%.[5] Besides racial types, sunlight exposure is essential to melasma development. Melasma is considered to be a cosmetic problem as there are no pain or other associated symptoms.[5,6]

Treatment of melasma involves the use of topical hypopigmenting agents such as hydroquinone, tretinoin, and azelaic acid and its derivatives.[7,8] A combination of hypopigmenting agents with different mechanisms of action has been found to be an efficacious treatment due to improved clinical efficacy and reduced duration of therapy as well as risk of adverse effects.[7,9] In this study, the combination of trans-4-(aminomethyl) cyclohexanecarboxylic acid (tranexamic acid [TXA])/potassium azeloyl diglycinate/niacinamide* was assessed for its clinical efficacy in improving hyperpigmented lesions. TXA is a synthetic derivative of lysine. It has been found that topical application of TXA can prevent ultraviolet radiation-induced hyperpigmentation of the dorsal skin of Weiser-Maples guinea pigs.[10] Its possible action is to block the release of prostaglandin, an activator of tyrosinase.[11] However, clinical reports on the topical use of TXA for treatment of melasma are currently limited. Potassium azeloyl diglycinate†

*Trademark: PEM-C (Pazana Laboratory Asia Co., Ltd, Bangkok, Thailand).

† Trademark: Azeloglicina® (Beijing Brilliance Biochemical Co., Ltd., Beijing, China).

(PAD) is a chemical modification of azelaic acid. Its mechanism of action has been found to be competitive inhibition of tyrosinase.[12,13] This molecule has been found to be better tolerated than azelaic acid.[12] The results of an efficacy study suggest that it improves skin brightness and reduces pigmentation.[14] Niacinamide, also known as nicotinamide, is the pyridine-3-carboxylic acid amide form of niacin, a component of vitamin B complex. It has been reported to inhibit melanosome transfer to keratinocytes in an in vitro study.[15] The results from a study in Japanese women indicated that 5% niacinamide significantly decreased hyperpigmentation and increased skin lightness compared with the vehicle alone after 4 weeks of use. According to the different actions on hypopigmentation of these compounds, it was expected that the synergistic effects of the combination would occur, resulting in an improvement in hyperpigmented lesions with no serious adverse events (AEs).

PATIENTS AND METHODS

Study Design

The design of this study was a randomized, double-blind and emulsion-based, controlled trial. The study was conducted at the Cosmetics and Natural Products Research Center, Naresuan University, Phitsanulok, Thailand, from April through July 2008, in patients with facial melasma. The study protocol was approved by an institutional review board of Naresuan University (Approval Code: 51 02 04 0010; Approval Date: February 26, 2008). A simple randomization scheme was used for allocation of eligible patients to the test or control groups. Random code (A or B) was prepared by independent staff by using a random table, and was concealed up to the time of allocation in sealed envelopes labeled with a unique patient number. These envelopes were opened sequentially after consent was obtained to enroll an eligible patient (at week 0 or before treatment). The product labeled with code A or B was then distributed to the patients by an independent staff member. Code allocation was concealed from all investigators, the dermatologist, and researchers until after data analysis was complete.

Study Population

In this study, the recruitment of patients was performed through advertising. Thai male and female patients with mild-to-moderate epidermal melasma, ranging from mild and discontinuous to moderate and homogeneous, as determined by a dermatologist were firstly recruited to the study. Patients were randomized for the application of the test product containing the combination of trans-4-(aminomethyl) cyclohexanecarboxylic acid/potassium azeloyl diglycinate/niacinamide. The improvement in hyperpigmentation after application was objectively evaluated by the relative melanin value (RMV) measured with a pigmentation/erythema measuring device (Mexameter MX 18, Courage and Khazaka Electronic GmbH, Köln, Germany). This value indicates the intensity of pigmentation relative to the surrounding normal skin.[16] Additionally, clinical evaluation by investigators through the Melasma Area and Severity Index (MASI) was performed. To be eligible for the study, patients had to have RMVs in the range of 20 to 120.[16,17] Subjects were excluded if they were a

smoker or alcoholic, a drug abuser, pregnant, nursing or planning to become pregnant, or had used topical steroids, hormones, antibiotics, NSAIDs, antihistamines, or medicated cosmetics containing alpha hydroxy acids, retinoids, azelaic acid, kojic acid, hydroquinone, chemical peels and/or other substances which might induce hypopigmentation on the face within 4 weeks of the start of the study; used systemic steroids, hormones, antibiotics, NSAIDs, antihistamines, or isotretinoin within 4 weeks of the start of the study; had a known allergy or sensitivity to any components contained in the product; or had any disease that might interfere with the evaluation of hyperpigmentation. All analyses were performed on the primary intent-to-treat (ITT) and on-treatment (OT) populations. The primary ITT population included all patients who had baseline assessment and received the product after randomization; missing data were handled by assigning the value recorded at the last patient visit. The OT population included those patients in the study who had an observational value and measurement for skin properties at that particular time point in the study.

STUDY PROTOCOL

At week 0 of the study, patients arrived at the study room at 8:00 AM. They then were asked to wash their face with clean water, pat the face dry with a towel, and wait for 30 minutes before proceeding to the next step of measuring the skin properties. Absolute melanin values at the hyperpigmented area and the normal area of each melasma location (forehead, right and left malar, and chin) were measured and RMV was determined. A mean of RMVs from 4 areas was used to indicate the intensity of pigmentation disorder in each patient. Other skin conditions including moisture content, pH, and redness (erythema value) were measured by using a skin hydration measurement device (Corneometer CM 825, Courage and Khazaka Electronic GmbH), pH measurement device (Skin-pH-Meter pH 900, Courage and Khazaka Electronic GmbH), and the previously mentioned pigmentation/erythema measurement device, respectively. The measurement room had a controlled temperature of 27±2°C and relative humidity of 55%±5%. Four measurements were taken of each facial area (forehead, right and left malar, and chin) that was the target site of product application. The mean of these 4 values indicated the skin moisture, pH, or redness of each patient. After completing baseline measurements, color photographs of patients were taken (EOS 400D with an EFS 18–55 mm lens, Canon Inc., Tokyo, Japan). Melasma severity of each patient was then scored using the MASI.[2,18,19] In the MASI system, the face is divided into 4 areas: forehead, right malar, left malar, and chin that correspond, respectively, to 30%, 30%, 30%, and 10% of total face area. The melasma in each of these areas was graded on 3 variables: percentage of total area involved on a scale from 0 (no involvement) to 6 (90%–100% involvement); darkness on a scale from 0 (absent) to 4 (severe); and homogeneity on a scale of 0 (minimal) to 4 (maximum). The MASI was then calculated with the following equation:

$$\text{MASI} = 0.3\,(\text{DF} + \text{HF})\,\text{AF} + 0.3\,(\text{DMR} + \text{HMR})\,\text{AMR} + 0.3\,(\text{DML} + \text{HML})\,\text{AML} + 0.1\,(\text{DC} + \text{HC})\,\text{AC},$$

where *D* was darkness, *F* was forehead, *H* was homogeneity, *A* was area, *MR* was right malar, *ML* was left malar, *C* was chin, and the values 0.3, 0.3, 0.3, and 0.1 were the respective percentages of total facial area.

The MASI grading of each patient was performed by 2 investigators (P.T. and J.V.). The investigators performed this grading independently and blind of each other's grading. Additionally, the MASI grading was assessed from photographs by one investigator (K.P.) who was blind to the MASI scores of the other investigators and to the patient's status whether it was before or after treatment. The mean from 3 values was used to indicate the melasma intensity of each patient. In this study, while assessing the photographs, details of the patient's face (eg, eyes, mouth) were concealed so that only the lesions were evaluated.

The enrolled patients then received either the test or the emulsion-based product. A personal diary for recording application time and adverse symptoms was also distributed to each patient.

Subjects were scheduled for study visits every 2 weeks to determine skin properties, including melanin and MASI values. Photographs of the patients were taken at each visit. AEs, including erythema, scaling, and edema, were also assessed by the dermatologist (P.T.) using the visual grading scale of Frosch and Kligman and COLIPA.[20–22] The scale ranged from 0 to 4 as follows: erythema, 0 = no evidence of erythema, 0.5 = minimal or doubtful erythema, 1 = slight redness, spotty, and diffuse, 2 = moderate and uniform redness, 3 = intense redness, and 4 = fiery redness; scaling, 0 = no evidence of scaling, 0.5 = dry without scaling or appears smooth and taut, 1 = fine or mild scaling, 2 = moderate scaling, and 3 = severe scaling with large flakes; and edema, 0 = absence of edema, and 1 = presence of edema. The same dermatologist assessed all enrolled patients.

Additionally, patients were interviewed by the same dermatologist if any AEs were apparent at each visit. Possible AEs were red skin, burning/stinging, itching, rash, papules, swelling, eczema, or blistering. The personal diaries are also examined for AEs recorded by the patients themselves.

Directions for Use of Products

Interventions of this study were 2 different products including the test (emulsion-base with the combination 6.5% trans-4-[aminomethyl] cyclohexanecarboxylic acid/potassium azeloyl diglycinate/niacinamide) and the control (emulsion-base alone). The emulsion-base consisted of polypropylene glycol-15 stearyl ether, isohexadecane, steareth-2, steareth-21, stearic acid, cetearyl alcohol, polyacrylamide, C13–14 isoparaffin, laureth-7, dimethicone, glycerine, phenoxyethanol, methylparaben, propylparaben, ethylparaben, butylparaben, isobutylparaben, sodium hyaluronate, and perfume. Both test and control products were prepared by Pazana Laboratory Asia Co., Ltd, and were similar in their physical characteristics and packaging.

Patients received written instructions and verbal explanations of the instructions. The assigned product was applied to melasma areas twice a day (at morning and at bedtime) after washing of the facial skin. The duration of application was 8 consecutive weeks. Patients were also provided with supplemental sunscreen product with

sun protection factor (SPF) 30 (Pazana Laboratory Asia Co., Ltd). The sunscreen agents incorporated in the sunscreen product were 4.00% w/w titanium dioxide and 2.32% w/w ethylhexyl methoxycinnamate. Other ingredients included butylene glycol cocoate, caprylic/capric triglycerides, isononyl isononanoate, dimethicone, cetyl dimethicone, candelilla/jojoba/rice bran polyglyceryl-3 ester (and) glyceryl stearate (and) cetearyl alcohol (and) sodium stearoyl lactylate, polysilicone-14, cetearyl alcohol, diglyceryl-2 diisostearate, glyceryl stearate SE, nicotinamide, magnesium aluminum silicate, shea butter, butyl methoxydibenzoylmethane, phenoxyethanol, ectoin, xanthan gum, tetrasodium EDTA, methylparaben, propylparaben, ethylparaben, butylparaben, isobutylparaben, and fragrance. Any moisturizer free from the agents specified in the exclusion criteria could be used after application of the test product or emulsion-base. Subjects were instructed not to take medication or apply topical medicine on the face during the study period. In addition, they were instructed not to apply any foundation, powder, or make-up to the face on the morning of a scheduled study visit, and avoid or minimize sun exposure and sunlamps during the study periods. The supplied sunscreen was applied to the face whenever sun exposure was anticipated, and wearing protective clothing was advised.

Determination of Subject Compliance

Patients were asked to return the used product and the sunscreen product while receiving the new one at each visit. The returned product was weighed by an investigator to monitor patient compliance.

Additionally, patients were interviewed by the dermatologist to determine if they were applying the product properly. The application time recorded in the personal diary was also examined.

Statistical Analysis

Descriptive statistics were used to report all results of this study in terms of mean (SD) and percentage (%). The mean differences of each of the skin parameters between treatments were analyzed using a 2-sample *t* test. $P < 0.05$ was considered statistically significant. Repeated-measures ANOVA was also used to compare mean RMV or MASI score at various time points during the administration of the product to the baseline value. The Bonferroni correction was performed to correct for multiple comparisons ($P = 0.05/4 = 0.0125$). Inter-rater reliability for MASI scored by an individual investigator was measured using the intraclass correlation coefficient (ICC). The Pearson correlation coefficient was used to evaluate the relation between the MASI and RMV.

RESULTS

Subject Disposition and Baseline Characteristics

Flow of patients through the study is shown in the **figure**. One hundred and thirty-nine Thai patients were assessed for enrollment and 67 patients were enrolled and randomized. The resulting primary ITT population included 33 patients in the test group and 34 patients in the control group. Of the primary ITT population, 60 patients

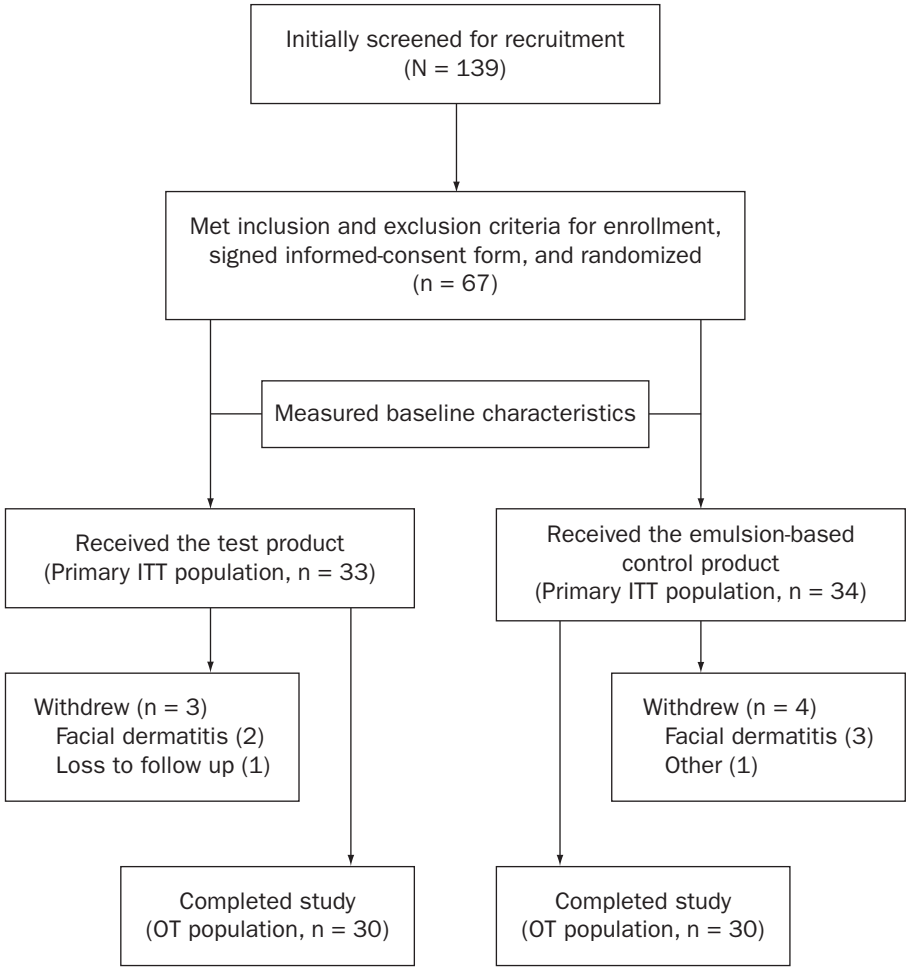

Figure. Subject disposition throughout the course of the study. ITT = intent-to-treat; OT = on-treatment.

completed all 8 weeks of the study: 30 of 33 patients (91%) in the test group and 30 of 34 patients (88%) in the control group.

Table I shows the demographic and mean baseline characteristics of the population. The enrolled patients ranged in age from 27 to 54 years (mean [SD], 42.2 [6.4] years) and were predominantly female (65 [97%]). The majority in both groups had melasma with RMVs in the range of 20 to 120 (test group, 79.1 [19.9] and control group, 75.3 [14.4]). There was not a statistically significant difference between groups in regard to age or any other demographic parameter. Patients in both groups were assessed by a dermatologist and reported as having no erythema, peeling, dryness, burning/stinging, or itching during the entire 8 weeks of treatment.

Table I. Demographic and baseline characteristics of patients enrolled in the study (N = 67).

Characteristic	Test Group (n = 33)	Control Group (n = 34)	*P**
Age, mean (SD), y	43.1 (6.3)	41.3 (6.4)	0.260
Sex, no. (%)			
Male	1 (3)	1 (3)	
Female	32 (97)	33 (97)	
Race, no. (%)			
Asian (Fitzpatrick skin type IV)	33 (100)	34 (100)	
Education, no. (%)			
Less than primary school	4 (12)	1 (3)	
Primary school	19 (58)	14 (41)	
High school	4 (12)	14 (41)	
Bachelor's degree or equivalent	6 (18)	5 (15)	
Occupation, no. (%)			
Government officer	2 (6)	1 (3)	
Contingent worker	9 (27)	19 (56)	
Agriculturalist	9 (27)	5 (15)	
Freelance/personal business	5 (15)	6 (18)	
Unemployed	8 (24)	3 (9)	
Skin properties, mean (SD)			
Moisture content, AU†	59.6 (7.8)	58.8 (8.2)	0.695
Skin pH	5.1 (0.5)	5.2 (0.5)	0.236
Erythema value, AU	339.0 (59.8)	337.1 (58.1)	0.899
RMV, AU	79.1 (19.9)	75.3 (14.4)	0.370
MASI score	17.3 (5.4)	15.9 (6.6)	0.351

AU = arbitrary unit; RMV = relative melanin value; MASI = Melasma Area and Severity Index.
*2-Group *t* test with a 2-sided significance level of 0.05.
† One unit represents a water content of stratum corneum of 0.02 mg/cm^2.

EFFICACY

Mean (SD) values of the measured parameter in the test and emulsion-based groups the primary ITT and OT populations are shown in **Table II**. In the OT population, the significant differences in mean RMV between the test (mean [SD], 63.1 [16.8] units) and the control (75.0 [15.5] units) groups were first observed at week 6 (P = 0.006). Focusing on the within-group results for the test product, a statistically significant decrease in RMV from the baseline was initially observed at week 4 (F = 13.92; P = 0.001). No significant within-group decreases from baseline in RMVs were noted at any visits in the control group. By the eighth week of treatment, a decrease in RMVs from baseline was observed in 25 test patients (83%) and 15 controls (50%).

Table II. Measured parameters in the test and control groups. Values are mean (SD).

Parameter	Primary ITT Population			OT Population		
	Test Group (n = 33)	Control Group (n = 34)	*P*	Test Group (n = 30)	Control Group (n = 30)	*P**
RMV, AU						
Week 0	79.1 (19.9)	75.3 (14.4)	0.370	80.6 (19.7)	74.4 (17.3)	0.220
Week 2	72.1 (16.2)	72.8 (15.9)	0.857	73.2 (16.2)	73.9 (17.3)	0.858
Week 4	67.9 (15.7)	73.6 (14.4)	0.123	68.5 (15.9)	74.7 (15.6)	0.130
Week 6	63.0 (16.4)	74.0 (14.2)	0.005	63.1 (16.8)	75.0 (15.5)	0.006
Week 8	59.6 (17.0)	74.7 (14.9)	<0.001	59.4 (17.4)	75.4 (16.3)	0.001
MASI score						
Week 0	17.3 (5.4)	15.9 (6.6)	0.351	17.7 (6.2)	16.0 (6.0)	0.275
Week 2	16.0 (4.7)	14.6 (6.3)	0.314	14.0 (3.6)	16.1 (4.9)	0.062
Week 4	14.7 (5.3)	14.8 (6.8)	0.962	12.7 (3.6)	16.1 (5.2)	0.005
Week 6	13.6 (4.7)	14.7 (5.8)	0.388	12.4 (3.2)	16.1 (4.1)	<0.001
Week 8	12.4 (3.7)	15.2 (6.3)	0.027	11.5 (2.7)	17.2 (4.3)	<0.001
Moisture content, AU†						
Week 0	59.6 (7.8)	58.8 (8.2)	0.695	43.1 (6.3)	41.3 (6.4)	0.403
Week 2	64.9 (8.5)	63.7 (7.2)	0.513	64.9 (8.3)	64.6 (9.6)	0.910
Week 4	66.8 (8.8)	67.7 (6.6)	0.667	67.0 (8.6)	68.0 (5.8)	0.560
Week 6	66.7 (8.4)	65.4 (8.4)	0.543	66.8 (8.1)	65.5 (8.1)	0.537
Week 8	66.9 (7.4)	67.6 (7.6)	0.699	67.1 (7.1)	68.0 (7.1)	0.611

(continued)

Table II (continued).

Parameter	Primary ITT Population			OT Population		
	Test Group (n = 33)	Control Group (n = 34)	*P*	Test Group (n = 30)	Control Group (n = 30)	*P**
Skin pH						
Week 0	5.1 (0.5)	5.2 (0.5)	0.236	5.1 (0.5)	5.3 (0.5)	0.115
Week 2	5.2 (0.6)	5.3 (0.5)	0.307	5.2 (0.6)	5.3 (0.5)	0.316
Week 4	5.4 (0.5)	5.5 (0.4)	0.452	5.4 (0.5)	5.5 (0.4)	0.431
Week 6	5.1 (0.5)	5.4 (0.5)	0.018	5.0 (0.5)	5.4 (0.6)	0.019
Week 8	5.1 (0.5)	5.1 (0.5)	0.893	5.0 (0.5)	5.0 (0.6)	0.824
Erythema value, AU						
Week 0	339.0 (59.8)	337.1 (58.1)	0.899	333.1 (53.7)	335.4 (53.3)	0.868
Week 2	339.3 (60.9)	337.2 (60.9)	0.888	334.0 (56.3)	334.6 (55.1)	0.966
Week 4	347.1 (62.8)	341.4 (60.3)	0.706	342.5 (59.2)	339.3 (54.6)	0.828
Week 6	345.8 (63.6)	342.6 (59.2)	0.836	341.0 (60.2)	340.7 (53.2)	0.982
Week 8	347.1 (58.8)	342.0 (64.2)	0.733	342.5 (54.6)	340.0 (59.4)	0.862

ITT = intent-to-treat; OT = on-treatment; RMV = relative melanin value; AU = arbitrary unit; MASI = Melasma Area and Severity Index.
*2-Group *t* test with a 2-sided significance level of 0.05.
† One unit represents the water content of stratum corneum of 0.02 mg/cm^2.

In the primary ITT population, based on the comparison between mean RMVs, significant differences were found at week 6 ($P = 0.005$) of the visits comparing the test (63.0 [16.4] units) and control (74.0 [14.2] units) groups. For the within-group comparison, a statistically significant decrease in mean RMV from baseline was initially found at week 4 ($F = 12.85$; $P < 0.001$) in the test group. No significant decreases in RMVs from the baseline were seen in the control group. After 8 weeks of treatment, decreases in mean RMV from baseline was observed in 25 (76%) and 15 (44%) patients enrolled in the test and emulsion-based groups, respectively.

The significant differences in mean MASI scores between the test and the control groups were initially observed at weeks 4 (test, 12.7 [3.6] vs control, 16.1 [5.2]; $P = 0.005$) and 8 (test, 12.4 [3.7] vs control, 15.2 [6.3]; $P = 0.027$) in the OT and primary ITT populations, respectively. For the within-group results with the test product, a statistically significant decrease in MASI scores from baseline was initially observed at weeks 2 (week 0, 17.7 [6.2] vs week 2, 14.0 [3.6]; $P = 0.006$) and 6 (week 0, 17.3 [5.4] vs week 6, 13.6 [4.7]; $P = 0.004$) in the OT and primary ITT populations, respectively. No significant decreases in MASI scores from the baseline were observed in the control group of the OT and ITT populations.

For data from the ITT population, the inter-rater reliability (ICC) of grading the MASI score by each investigator ranged from 0.86 to 0.88. The Pearson correlation coefficient suggested a proportional relationship between mean RMV and mean MASI scores of each visit (weeks 0, 2, 4, 6, and 8) and this relationship was significant (Pearson correlation = 0.714; $P = 0.020$).

In both ITT and OT populations, treatment with either the test or the emulsion-based product caused a gradual increase (from week 0 to week 4) in skin pH. However, skin pH approached that of the baseline value after 6 weeks of treatment. Mean differences in erythema values between the test and the control groups were not statistically significant for all measurements.

Tolerability

The AEs which occurred in patients are summarized in **Table III**. Overall, AEs were reported by 4 (12%) and 5 (15%) patients in the test and control groups, respectively. The majority of AEs were burning and/or stinging. During the entire study period, 5 patients (7%) in the primary ITT population discontinued treatment due to AEs. In the test group, 2 patients (6%) experienced AEs that led to withdrawal. Three patients (9%) withdrew from the control group due to AEs. One patient from both groups reported an intense redness event. The intense redness coupled with moderate scaling was observed in one patient from the test group. One patient in the control group experienced intense erythema with edema at week 4. All patients recovered spontaneously when they discontinued using the products. No other serious AEs were reported.

Patient Compliance

The compliance of patients was monitored by weighing the products before and after the study period and checking the frequency of application of the distributed products during study periods. The data indicated that an average of 12.4 (0.8) g of

Table III. Adverse events (AEs) observed or reported in the test and control groups during the study. All data are number (%).

AE	Test Group (n = 33)	Control Group (n = 34)	*P**
Graded by dermatologist			
Erythema			
Slight redness	1 (3)	2 (6)	0.558
Moderate redness	0	1 (3)	0.315
Intense redness	1 (3)	1 (3)	0.987
Scaling			
Dry without scaling	1 (3)	0	0.318
Moderate scaling	1 (3)	0	0.313
Edema	0	1 (3)	0.331
Reported by patients			
Burning and/or stinging	4 (12)	5 (15)	

*2-Group *t* test with a 2-sided significance level of 0.05.

the control or 12.1 (0.6) g of the test product was used per person over 2 weeks and a mean of 30.2 g (0.6) of the sunscreen was used over 4 weeks. By interviewing the patients and assessing their diaries, it was determined that all patients followed the instructions of product application. They also used the sunscreen during periods of ultraviolet (UV) exposure and/or avoided sun exposure by wearing protective clothes or a hat.

DISCUSSION

The purpose of this study was to assess the efficacy of a formulation containing a combination of trans-4-(aminomethyl) cyclohexanecarboxylic acid/potassium azeloyl diglycinate/niacinamide in the treatment of melasma in Thai adults. RMV was used as a primary outcome for indicating the improvement of hyperpigmentation because this value objectively reflects the intensity of pigmentation. The primary ITT analysis of patients with available data showed that significant differences in mean RMV between the 2 treatment groups were observed after 6 weeks of treatment. A significant decrease in mean RMV from baseline was observed after 4 weeks in the test group and no significant reduction in RMV was noted in the control group. Similar results were found for the OT analysis. Additionally, in both primary ITT and OT populations, a higher proportion of patients in the test group showed a decrease in mean RMV from baseline compared with the control group. These findings indicate that the test product containing the combination was associated with objective reduction of melasma intensity in Thai adults.

Besides mean RMV, the intensity of melasma was assessed from the visual MASI scored by an individual investigator. ICC in the range of 0.86 to 0.88 indicated the reliability of a visual scoring process. Additionally, according to the Pearson correlation analysis, the proportional relationship between mean RMV and mean MASI scores

of each visit indicated that the reduction of mean RMV measured using an instrumental method was aligned with the reduction of melasma intensity observed by investigators. This also implies that such melasma reduction was objectively relevant and perceptible. However, it should be noted that there were differences between results from ITT and OT analysis. OT analysis suggested a significant difference in melasma intensity graded by visualization between the test and control groups at week 4, whereas ITT analysis indicated a significant difference of melasma intensity at week 8. Within-group analysis provided a statistically significant decrease in MASI scores from the baseline at weeks 2 and 6 in the OT and primary ITT populations, respectively. These differences may involve exclusion of patients with large intensity and size of melasma, so that a substantial improvement was seen by visualization in the OT population with lower intensity.

In the present study, the clinical improvement of melasma in the test group might result from interference in different steps of the melanogenesis pathway by the active agents contained in the combination. TXA might suppress melanogenesis caused by UV light exposure by inhibition of synthesis of arachidonic acid and/or prostaglandins,[10,11,23] which are the mediators of melanocyte stimulation. Together with tyrosinase inhibitory activity of PAD[12] and melanosome transfer inhibitory activity of niacinamide,[14] the melanogenesis inhibitory activity would be enhanced. In addition, the synergistic beneficial effect of the combination might be enhanced by using the sunscreen with SPF 30 throughout the study period.

One more expectation from use of the combination is to reduce the risk of adverse effects. The test product was tolerated well during the study period of 8 weeks. Type and frequency of AEs were consistent with azelaic acid in the treatment of melasma as the irritant effects were mild and transient.[7] A multicenter, randomized, double-masked, parallel-group study assessed the efficacy, safety, and tolerability of azelaic acid 20% cream compared with those of its vehicle for the treatment of facial hyperpigmentation in darker-skinned patients. The mean severity scores of AEs after treatment with 20% azelaic acid for 24 weeks were <1 (below trace levels).[24] In the present study, all AEs are unlikely to be from the combination as the incidences occurred in both treatment groups. Moreover, the proportions of patients with AEs in the test (4 [12%]) and control (5 [15%]) groups were not significantly different. Skin pH of test or emulsion-based group gradually increased from week 0 to week 4. However, it was closer to the baseline value (5.1–5.3) after 6 weeks of treatment in both groups. In general, normal skin pH is in the acidic range (between 4.0–6.5) which acts as a buffer against alkaline irritants, whereas many forms of eczema cause an alkaline shift in the skin pH.[25] Therefore, skin surface pH measurement can be used to indicate subclinical eczema in irritation tests and evaluation therapy.[26] Besides skin pH, the tolerability to the products could confirm with the erythema values that did not significantly change in all measurements in comparison with the baseline value.

A limitation of this study was the exclusion criteria that limited the ability to extrapolate the results to the group of majority men, smokers, alcohol drinkers, and/or birth control pill users. The results from a small number of patients within this study may also not coincide with a larger study with a variety of population and skin types.

In addition, a small number of patients together with a short duration of follow-up (8-week study) may lead to a relative lack of power and a limited ability to detect potentially important differences in clinically relevant AEs. Future studies with a larger number of patients and longer duration should be performed to ensure the beneficial effects of the combination formulation for melasma treatment.

CONCLUSIONS

The significant differences in RMVs between the test and control groups were observed after 6 weeks of treatment, both in the primary ITT and OT populations. The incidence of patients with AEs was not significantly different between the test and control groups.

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