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ORIGINAL RESEARCH ARTICLE

Efficacy and Safety of Fluocinolone Acetonide, Hydroquinone, and Tretinoin Cream in Chinese Patients with Melasma: A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Parallel-Group Study

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Abstract

Background and Objectives This study aimed to determine the efficacy and safety of fluocinolone acetonide, hydroquinone, and tretinoin (FAHT) cream for the treatment of moderate and severe facial melasma. The primary objective was assessment of clinical efficacy, instrumental measured efficacy, and integral therapeutic efficacy at the end of weeks 4 and 8.

Methods A total of 233 subjects were randomly allocated (1:1 ratio) to receive topically administered FAHT cream ($n = 117$) or placebo ($n = 116$) once nightly for 8 weeks. Observed side effects were documented throughout.

Results In the per protocol set (PPS; those subjects who met all requirements of the protocol), the integral therapeutic efficacy rate of FAHT cream on moderate and severe melasma was 68.57 % (vs. placebo, 0.94 %), the clinical effective rate of FAHT cream was 74.29 % (vs. placebo, 0.94 %), and the instrumental measure efficacy of FAHT cream was 71.43 % (vs. placebo, 6.60 %). The difference in efficacy between the two groups was statistically significant ($p < 0.001$). In the full analysis set (FAS; the PPS and those subjects who were lost to follow-up but received at least one study treatment), the integral therapeutic efficacy rate of FAHT cream was 64.60 % (vs. placebo, 0.88 %), the clinical effective rate of FAHT cream was 69.91 % (vs. placebo, 0.88 %), and the instrumental measure efficacy of FAHT cream was 69.03 % (vs. placebo, 7.08 %). The difference in efficacy between the two groups was statistically significant ($p < 0.001$). Of 113 subjects in the FAHT group, 34 (30.1 %) reported adverse effects. Most of the pathological adverse effects were mild and resolved with either continuous treatment or discontinuation. Of 113 subjects in the placebo group, three (2.6 %) reported mild adverse effects. No severe adverse effects or other abnormal clinical results were associated with the study treatment.

Conclusion FAHT cream is efficacious, well tolerated, and has a high margin of safety for the treatment of moderate and severe melasma in the Chinese population.

China Food and Drug Administration (CFDA) Trial Registration Number: 2008L01344.

Z. Gong and W. Lai contributed equally to this article.

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Key Points

The efficacy of fluocinolone acetonide, hydroquinone, and tretinoin (FAHT) cream is superior to placebo for the treatment of moderate and severe melasma.

Treatment of moderate and severe melasma with FAHT is associated with a high margin of safety and high tolerance.

1 Introduction

Melasma is a common acquired symmetric hypermelanosis characterized by irregular light- to grey-brown macules and patches involving sun-exposed areas of skin; it mostly affects women of reproductive age [1–3]. A recent report of survey results in nine countries indicates that 41 % of females developed melasma after pregnancy but before menopause [1, 4–6]. More than half of Asian melasma patients are seeking esthetic help because even mild melasma can disturb outdoor social activities and dampen emotional and psychological well-being [1, 7, 8].

Melasma is often a therapeutically challenging disease because it is refractory, prone to relapse, and characterized by incomplete clearance, frequent remission, and rebound hyperpigmentation. Current treatments include hypopigmenting agents, such as topical hydroquinone, along with broad spectrum (UVA/UVB) sunscreen, chemical peels, and laser treatments [1–3]. A combination of multiple therapeutic methods is superior to monotherapy. The use of hydroquinone alone, either in low concentration over-the-counter (2 %) formulation or high concentration (4 %) as prescribed, is not recommended to treat melasma except as maintenance therapy [1, 9]. Other hypopigmenting or lightening agents include retinoic acid (tretinoin) and azelaic acid. The ability of kojic acid, isopropylcatechol, *N*-acetyl-4-cysteaminylphenol, and flavonoid extracts to produce hypopigmentation is being investigated; pending further studies of efficacy and safety, these compounds are not recommended. Alternatively, chemical peels, laser treatments, and intensive pulsed-light therapy are considered additional therapeutic modalities for treating melasma [1–3].

In 1975, Kligman and Willis first proposed 'Kligman's Formula', the combination of hydroquinone, tretinoin, and dexamethasone acetate (cortical steroid hormones) for the external treatment of melasma [9, 10]. Topical application of the combination hydrophilic cream (5 % hydroquinone, 0.1 % tretinoin, and 0.1 % dexamethasone) for 3 months was effective for the treatment of melasma, while the use of hydroquinone, tretinoin, or dexamethasone alone failed to diminish freckles. In addition, Kligman and Wallis [9] found that tretinoin facilitates proliferation and differentiation of epithelial cells, as well as keratolysis in laboratory experiments, thereby eliminating atrophoderma caused by topically administered cortical steroid hormones. In 2002, the US FDA approved Tri-Luma® to treat moderate and severe melasma.

The components and formulation of fluocinolone acetonide, hydroquinone, and tretinoin (FAHT) cream (Zhejiang Rishengchang Pharmaceutical Co., Ltd, Dongyang, China) are identical to that of the US-approved Tri-Luma®. This product is formulated as oil-in-water and is supplied in 20-g tubes with 4 % hydroquinone, 0.05 % tretinoin, and 0.01 % fluocinolone acetonide as active ingredients. The benefits of FAHT cream are based on the formulary balance of the three active ingredients and the unique roles of the individual components [11].

Hydroquinone, a standard decolorizing agent, reduces the synthesis of melanin by inhibiting the activity of tyrosinase. Tretinoin also has some ability to inhibit tyrosinase activity, and replaces existing melanin by facilitating epithelial cell metabolism and regeneration by inducing DNA synthesis in epithelial cells and hypodermal cells. In addition, the effect of tretinoin on keratolysis is conducive for hydroquinone diffusion into the epithelial cell layer. Therefore, tretinoin thickens the skin to compromise atrophoderma caused by cortical steroid hormones. Fluocinolone in this compound formulation not only inhibits the synthesis of melanosomes by decreasing basic cellular activity but also reduces hydroquinone-induced skin irritation and inflammation to alleviate adverse effects.

To evaluate the efficacy and safety of FAHT cream for the treatment of moderate and severe facial melasma, a randomized, double-blind, placebo-controlled study of parallel groups was conducted in multiple sites following approval by the China FDA (Trial Registration No. 2008L01344).

2 Patients and Methods

2.1 Patients

Informed consent was obtained from all individual participants included in the study. Clinical criteria of diagnosis were based on the Diagnostic and Treatment Criteria of Melasma and Leukoderma of the Chinese Society of Integrated Traditional Chinese and Western Medicine:

1. Symmetrical appearance of clearly bounded patches of light brown to dark brown color with no inflammation or scar.
2. No obvious symptoms, such as itching or pain.
3. Frequently occurs in females, and primarily after adolescence.
4. The extent of disease may be seasonal, and is often greater in summer and lesser in winter.
5. Other chromatosis diseases, such as nevus fusco-careuleus zygomaticus or Riehl melanosis, can be excluded.

All patients with melasma according to the above diagnostic criteria were subject to the following criteria in order to participate in the study; other patients not meeting these criteria were excluded.

1. Male and female patients 18–65 years of age.
2. Baseline total target score (TTS) $\geq$4 (TTS = darkness score of damaged skin + area score of damaged skin).
3. Able to take medicine as required and come to the hospital for scheduled clinical assessments, instrument measurements, and digital photography.
4. Able to maintain regular work, study, and lifestyles and to avoid excessive sun exposure.
5. Willing and able to give informed consent.

To meet the regulatory requirements for a new drug application (NDA), at least 100 pairs of qualified patients were included. A dropout rate of 20 % was expected; therefore, the target sample size for enrollment was 240. Of 237 patients screened, 233 were enrolled in the study and 4 were excluded. Enrolled patients were randomly allocated into the test group or the control group. Twenty-four patients in each group were assessed in each study site.

2.2 Randomization and Blind Treatment

Prior to study initiation, a stratified randomization schedule with a 1:1 allocation was employed based on the Microsoft Excel 2003 VBA RND function (Microsoft Corporation, Redmond, WA, USA) programmed by the Department of Medical Statistics and Epidemics at Sun Yat-sen University. In order of enrollment, subjects were sequentially assigned a unique serial number that directed the administration of FAHT cream or placebo. Study subjects and investigators were blinded to the group allocation results.

2.3 Determination of Administration Methods

The dosage, administration route, and dosing regimen were identical for both the test group and the control group. An amount of study drug approximately the size of a grain of rice was applied to an area of skin approximately 2.5 cm in diameter. Subjects were instructed to apply the study drug on the affected area each night before bedtime for 8 weeks, after thoroughly cleansing the skin, and to avoid contaminating the mucous area between the eyes, nose, and mouth. Subjects were also instructed to apply appropriate sunscreen, such as SPF30, to the area of the face being treated.

2.4 Study Visits

Candidates were screened for a period of 1 week prior to double-blinded allocation to the FAHT group or the placebo group. After dosing, all subjects attended follow-up visits at weeks 2, 4, and 8. The last day of weeks 4 and 8 were the minor and major timepoints, respectively, to determine effectiveness.

2.5 Assessments

The darkness of each damaged skin area was assessed and scored as follows: 0 = normal skin; 1 = light brown (slightly different from the neighboring normal skin); 2 = brown (different from the neighboring normal skin); 3 = dark brown (significantly different from the neighboring normal skin).

The maximum length and maximum width of each damaged skin area was assessed and scored as follows: 0 = no skin damage; 1 = area of the damaged skin $\leq$2 cm^2; 2 = area of the damaged skin = 2–4 cm^2; 3 = area of the damaged skin $\geq$4 cm^2.

The TTS of a lesion was determined by the sum of the darkness and area scores of damaged skin: TTS = darkness score of damaged skin + area score of damaged skin.

2.5.1 Measurement of Melanin in Targeted Lesions (ΔMelanin)

A narrow band reflectance spectrophotometer (Mexameter MX18; Courage + Khazaka electronic GmbH, Cologne, Germany) was employed to measure the melanin values of damaged skin and the surrounding area (0.5–1 cm distance, approximately 2 cm^2 total area) normal skin. Thus, the Δmelanin value was calculated as follows:

$$\Delta\text{melanin} = \text{melanin of damaged skin} - \text{melanin of normal skin.}$$

2.5.2 Observation of Adverse Reactions

At each follow-up visit during the study, any reported potential adverse reactions were documented in as much detail as possible. Special attention was paid to the following adverse reactions: erythema, edema, exudation,

papules, itching, pain, and tingling. Detailed clinical signs and symptoms, locations, timing, duration, degree of severity (mild, moderate, severe), likelihood of relation to the test article (unlikely, possible, probable, likely, definitely), weight of evidence, mode of action (discontinuation, continuous administration, and assistant medication), and prognosis were promptly determined. In the event of severe adverse reactions, such as death, life-threatening illness, permanent or temporary organ damage and/or malfunctions or dysfunctions, and immediate or deferred hospitalization, administration of the study treatment was to be terminated immediately.

The adverse reactions were ranked in three different levels of severity.

1. Mild: does not affect daily life.
2. Moderate: affects daily life.
3. Severe: significantly affects daily life.

2.6 Study Objectives

The primary objectives were clinical efficacy, instrumental measured efficacy, and integral therapeutic efficacy, as assessed at the end of weeks 4 and 8. Integral therapeutic efficacy is a measure of cases being cured, greatly improved, improved, or unchanged.

2.6.1 Clinical Efficacy

$$\text{Decreased Index of Total Target Score (DITTS)} = \frac{(\text{TTS before treatment} - \text{TTS after treatment})}{\text{TTS before treatment}}$$

Criteria for clinical efficacy were ranked in four different grades.

1. Cured: DITTS $\geq$0.8.
2. Greatly improved: $0.5 \leq$ DITTS < 0.8.
3. Improved: $0.3 \leq$ DITTS < 0.5.
4. No change: DITTS $<$0.3.

2.6.2 Instrumental Measured Efficacy

$$\text{Improvement rate of target skin melanin (IRTSM)} = \frac{(\Delta\text{melanin before treatment} - \Delta\text{melanin after treatment})}{\Delta\text{melanin before treatment}} \times 100\%$$

Criteria for the instrumental measured efficacy were ranked in four different grades.

1. Cured: IRTSM $\geq$90 %.
2. Greatly improved: 60 %$\leq$ IRTSM $<$ 90 %.
3. Improved: 30 % $\leq$ IRTSM $<$ 60 %.
4. No change: IRTSM $<$30 %.

2.6.3 The Integral Therapeutic Efficacy

Criteria for the integral therapeutic efficacy were ranked in four different grades:

Cured: DITTS $\geq$0.8 and IRTSM $\geq$90 %.
Greatly improved: DITTS $\geq$0.5 and IRTSM $\geq$60 %.
Improved: DITTS $\geq$0.3 and IRTSM $\geq$30 %.
No change: DITTS $<$0.3 or IRTSM $<$30 %.

2.7 Statistical Analysis

Statistical analysis was performed using SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA), and various types of diseases were compared by superiority trials. Chi-square values and rank sum values were compared using two-tailed tests. A p value of $\leq$0.05 was considered statistically significant.

The datasets of statistical analysis included a per protocol set (PPS), full analysis set (FAS), and safety analysis set (SAS). The PPS included those subjects who met all protocol requirements. The FAS included the PPS and those subjects who were lost to follow-up but received at least one study treatment. The last observations of subjects lost to follow-up were carried forward (LOCF). The SAS included the study subjects who used the study treatment, attended at least one follow-up visit, and provided partial safety data. In this study, the baseline data were analyzed in the FAS, and the efficacy data were analyzed in the PPS and FAS (although period effectiveness was evaluated in the PPS only, and safety evaluated in the SAS only).

Baseline quantitative data were expressed as mean $\pm$ standard deviation (SD), and comparisons between the two groups were conducted using Student's t test. The grouping data were expressed as tabulated frequency and percentage, and comparisons between the two groups were conducted using the Chi-square test. The data beyond the Chi-square test were analyzed using Fisher's exact test.

Therapeutic evaluations of clinical efficacy, the instrumental measured efficacy, and the integral therapeutic efficacy were expressed as tabulated frequency and percentage. The ridit analysis results for the four graded scores between two groups were expressed as the weighted mean difference (WMD) of efficacy. If the difference between the lower and upper limits of the 95 % confidence interval (95 % CIL and 95 % CIU, respectively) did not contain 0, then $p \leq 0.05$; effective rates were expressed as tabulated frequencies and percentages. The two groups were analyzed using Cochran–Mantel–Haenszel (CMH) stratified analysis (Breslow–Day) with a Chi-square test.

The period efficacy as determined by investigator assessment of the decreased melanin index of total scores and the improved rate was expressed as median and

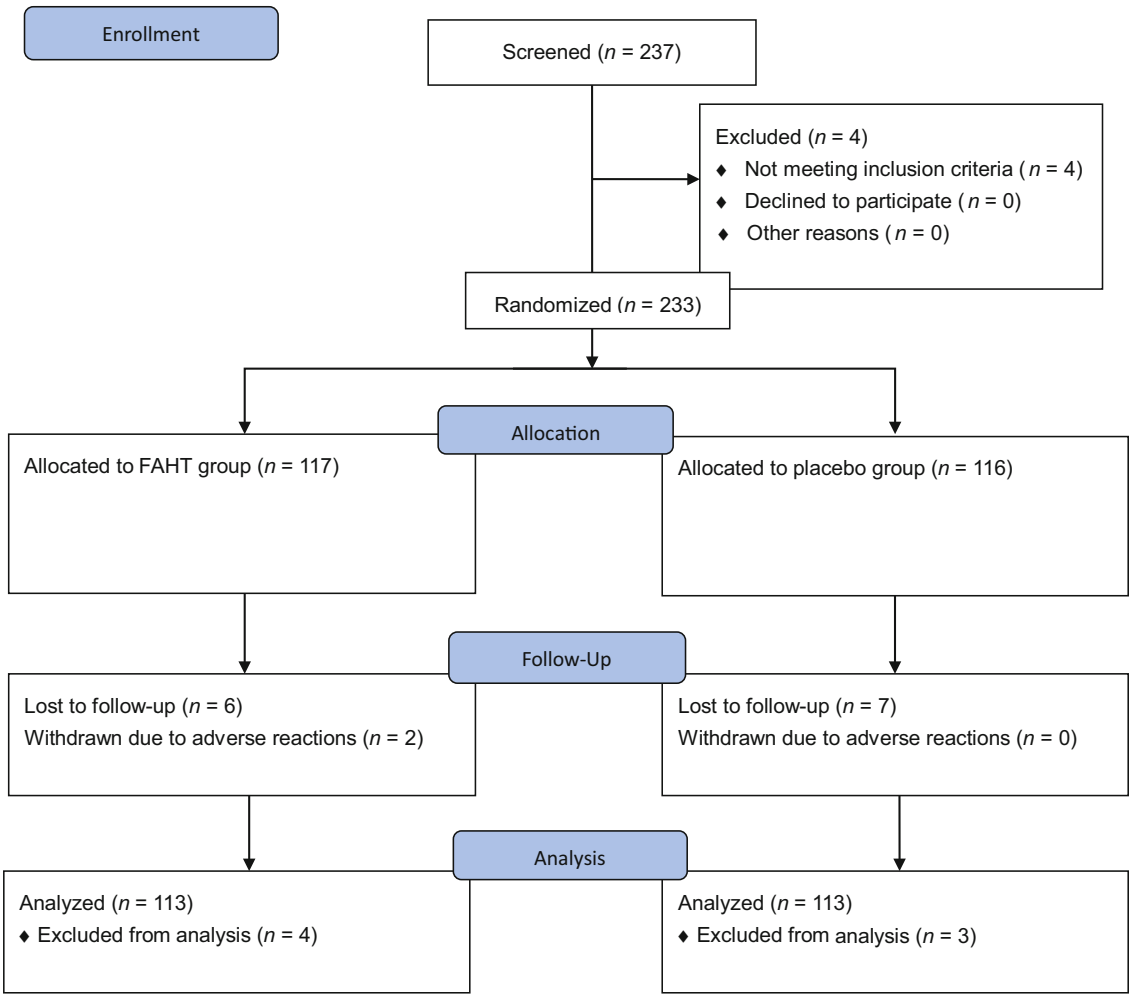

Fig. 1 Patient allocation and analysis. *FAHT* fluocinolone acetonide, hydroquinone, and tretinoin

mean ± SD, and analyzed using the rank-sum test for two-group comparison.

The occurrence/incidence of adverse reactions in safety evaluation and the comparison of laboratory tests were expressed as tabulated frequencies and percentages. Comparison between two groups was analyzed using the Chi-square test. The data beyond the Chi-square test were analyzed using Fisher's exact test.

3 Results

3.1 Baseline Characteristics

A total of 233 patients were enrolled in the study (FAHT group, $n = 117$; placebo group, $n = 116$), with only 226 patients completing the study ($n = 113$ for each group). The remaining subjects were lost to follow-up or withdrew because of adverse events. The PPS was satisfied with 105 patients in the FAHT group and 106 patients in the placebo group. The SAS included 113 patients in each group and was sufficient for safety and tolerance evaluation (Fig. 1).

No significant differences were found between the two groups in baseline values and demographic characteristics. Compliance was defined as taking more than 80 % of the total amount of medicine actually administered; subjects taking less than this amount were considered noncompliant. No significant differences were observed in compliance between the two groups (Table 1).

3.2 Assessments for Calculation of Clinical Efficacy

Marked significant differences were observed in DITTS and IRTSM between the FAHT treatment group and the placebo group (Table 2). After treatment for 2, 4, and 8 weeks, the DITTS values of target lesions were greater in the FAHT group (0.18, 0.31, and 0.51, respectively) than in the placebo group (0.03, 0.07, and 0.10, respectively). Values of IRTSM at 2, 4, and 8 weeks were greater in the FAHT treatment group (32.76, 47.06, and 64.49,

Table 1 Demographic baseline, and safety characteristics

	FAHT group	Placebo group	Statistical analysis
Patients [*n*]	113	113	
Sex [*n*]			
Male	2	0	
Female	111	113	
			$\chi^2 = 2.018$, $p = 0.498$
Age, years			
Range	20.55–59.72	22.12–61.14	
Mean ± SD	40.33 ± 7.26	41.29 ± 8.35	
			$T = 0.920$, $p = 0.359$
Disease duration, months			
Range	2–360	1–384	
Mean ± SD	81.78 ± 76.02	70.73 ± 63.11	
			$T = -1.188$, $p = 0.236$
Lesions [*n* (%)]			
Forehead	2 (1.71)	7 (6.19)	
Left zygomaticus	39 (34.51)	42 (37.17)	
Right zygomaticus	52 (6.02)	42 (37.17)	
Left buccal	10 (8.85)	15 (13.27)	
Right buccal	10 (8.85)	7 (6.19)	
			$\chi^2 = 5.482$, $p = 0.241$
Baseline total target score			
Range	4–6	4–6	
Mean ± SD	5.51 ± 0.63	5.35 ± 0.63	
			$T = -1.910$, $p = 0.057$
Baseline △melanin			
Range	3–163	16–185	
Mean ± SD	67.28 ± 32.65	66.19 ± 35.57	
			$T = -0.240$, $p = 0.810$
Compliant [*n* (%)]	108 (95.58)	105 (92.92)	
Noncompliant [*n* (%)]	5 (4.42)	8 (7.08)	
			$\chi^2 = 0.735$, $p = 0.391$
Length of exposure to study drug or placebo (days)			
Range	14–63	15–62	
Mean ± SD	53.96 ± 9.12	54.80 ± 7.65	
			$T = 0.751$, $p = 0.454$

FAHT fluocinolone acetonide, hydroquinone, and tretinoin cream, *SD* standard deviation

respectively) than in the placebo group (6.11, 13.44, and 17.74, respectively).

3.2.1 Major Efficacy Analysis (Week 8)

The clinical effective rate of FAHT cream on moderate and severe melasma for 8 weeks in the PPS was 74.29 % compared with a clinical effective rate of 0.94 % for the placebo (Table 3), a difference of 41 % as demonstrated by ridit analysis. The clinical effective rate of FAHT cream was 69.91 % in the FAS compared with an effective rate of 0.88 % for the placebo, a significant difference of 38 % as demonstrated by ridit analysis (Table 3).

In the PPS, the instrumental measured effective rate of FAHT cream on moderate and severe melasma for 8 weeks by the instrumental measurement was 71.43 % compared with an effective rate of 6.6 % for the placebo, a difference of 37 % as demonstrated by ridit analysis. In the FAS, the effective rate of FAHT cream was 69.03 % compared with an effective rate of 7.08 % of the placebo, a significant difference of 35 % as demonstrated by ridit analysis (Table 3).

Table 2 Assessments for calculation of clinical efficacy and instrumental measured efficacy

	FAHT group	Placebo group	Statistical analysis[a]
Decreased Index of Total Target Score (DITTS)			
Patients [*n*]	105	106	
Week 2			
Median	0.18	0.03	
Range	0.00–0.60	0.00–0.33	
Mean ± SD	0.18 ± 0.14	0.04 ± 0.08	
			$Z = -7.899$, $p = 0.000$
Week 4			
Median	0.31	0.07	
Range	0.00–1.00	0.00–0.50	
Mean ± SD	0.31 ± 0.19	0.08 ± 0.12	
			$Z = -8.856$, $p = 0.000$
Week 8			
Median	0.51	0.10	
Range	0.00–1.00	−0.20 to 0.50	
Mean ± SD	0.48 ± 0.21	0.10 ± 0.14	
			$Z = -10.614$, $p = 0.000$
Improvement rate of target skin melanin (IRTSM)			
Patients [*n*]	105	106	
Week 2			
Median	32.76	6.11	
Range	−350.52 to 466.67	−143.75 to 78.59	
Mean ± SD	30.00 ± 66.60	3.56 ± 34.29	
			$Z = -5.588$, $p = 0.000$
Week 4			
Median	47.06	13.44	
Range	−1100.00 to 225.00	−199.18 to 79.78	
Mean ± SD	35.00 ± 125.50	10.91 ± 39.19	
			$Z = -6.789$, $p = 0.000$
Week 8			
Median	64.49	17.74	
Range	−333.33 to 234.09	−100.00 to 85.25	
Mean ± SD	56.14 ± 66.56	16.20 ± 32.89	
			$Z = -8.352$, $p = 0.000$

FAHT fluocinolone acetonide, hydroquinone, and tretinoin cream, *SD* standard deviation

[a] *Z* sum of the group rank

Analysis of the PPS showed an integral therapeutic efficacy of 68.57 % for 8 weeks of treatment of FAHT cream for moderate and severe melasma compared with an integral therapeutic efficacy of 0.94 % for the placebo. This corresponds to a 39 % difference in clinical efficacy between the two groups as demonstrated by ridit analysis. In the FAS, the integral therapeutic efficacy of FAHT cream was 64.6 % compared with an integral therapeutic efficacy of 0.88 % for the placebo, a significant 35 % difference as demonstrated by ridit analysis (Table 3).

3.2.2 Minor Efficacy Analysis (Week 4)

The clinical effective rate of FAHT cream on moderate and severe melasma for 4 weeks in the PPS was 20 % compared with a clinical effective rate of 0.94 % for the placebo (Table S1 in the electronic supplementary material [ESM]), a difference of 25 % as demonstrated by ridit analysis. The clinical effective rate of FAHT cream was 19.47 % in the FAS compared with an effective rate of 0.88 % for the placebo, a significant difference of 24 % as demonstrated by ridit analysis (Table S1 in the ESM).

Table 3 Analysis of integral therapeutic efficacy at week 8

	PPS		FAS	
	FAHT group	Placebo group	FAHT group	Placebo group
Analysis of clinical efficacy by Decreased Index of Total Target Score (DITTS)				
Patients [*n*]	105	106	113	113
4-grade rank [*n* (%)]				
Cured	5 (4.76)	0 (0)	5 (4.42)	0 (0)
Greatly improved	73 (69.52)	1 (0.94)	74 (65.49)	1 (0.88)
Improved	12 (11.43)	13 (12.26)	13 (11.50)	14 (12.39)
No change	15 (14.29)	92 (86.79)	21 (18.58)	98 (86.73)
Ridit analysis				
Mean ± SD	0.70 ± 0.20	0.30 ± 0.11	0.69 ± 0.22	0.31 ± 0.12
WMD ± SD	0.41 ± 0.00		0.38 ± 0.00	
95 % CI	0.42–0.40		0.39–0.38	
Effective rate analysis				
Effective rate [%]	74.29	0.94	69.91	0.88
χ^2 (*P*)	121.140 (0.000)		117.721 (0.000)	
CMH test				
χ^2 (*P*)	3.407 (0.492)		3.656 (0.455)	
Analysis of instrumental measured efficacy by improvement rate of target skin melanin (IRTSM)				
Patients [*n*]	105	106	113	113
4-grade rank [*n* (%)]				
Cured	17 (16.19)	0 (0)	17 (15.04)	0 (0)
Greatly improved	58 (55.24)	7 (6.60)	61 (53.98)	8 (7.08)
Improved	15 (14.29)	22 (20.75)	15 (13.27)	23 (20.35)
No change	15 (14.29)	77 (72.64)	20 (17.70)	82 (72.57)
Ridit analysis				
Mean ± SD	0.68 ± 0.23	0.32 ± 0.17	0.67 ± 0.24	0.33 ± 0.17
WMD ± SD	0.37 ± 0.00		0.35 ± 0.00	
95 % CI	0.37–0.36		0.35–0.34	
Effective rate analysis				
Effective rate [%]	71.43	6.60	69.03	7.08
χ^2 (*P*)	93.295 (0.000)		91.977 (0.000)	
CMH test				
χ^2 (*P*)	4.225 (0.376)		4.137 (0.388)	
Analysis of integral therapeutic efficacy				
Patients [*n*]	105	106	113	113
4-grade rank [*n* (%)]				
Cured	5 (4.76)	0 (0)	5 (4.42)	0 (0)
Greatly improved	67 (63.81)	1 (0.94)	68 (60.18)	1 (0.88)
Improved	13 (12.38)	9 (8.49)	13 (11.50)	10 (8.85)
No change	20 (19.05)	96 (90.57)	27 (23.89)	102 (90.27)
Ridit analysis				
Mean ± SD	0.69 ± 0.22	0.31 ± 0.10	0.68 ± 0.23	0.32 ± 0.11
WMD ± SD	0.39 ± 0.00		0.36 ± 0.00	
95 % CI	0.39–0.38		0.37– 0.35	
Effective rate analysis				
Effective rate [%]	68.57	0.94	64.60	0.88
χ^2 (*P*)	106.618 (0.000)		104.159 (0.000)	
CMH test				
χ^2 (*P*)	3.353 (0.501)		3.572 (0.467)	

CI confidence interval, *CMH* Cochran–Mantel–Haenszel, *FAHT* fluocinolone acetonide, hydroquinone, and tretinoin cream, *WMD* weighted mean difference, *SD* standard deviation, *PPS* per protocol set, *FAS* full analysis set

In the PPS, the instrumental measured effective rate of FAHT cream on moderate and severe melasma for 4 weeks using the instrumental measurement was 35.24 % compared with an effective rate of 5.66 % for the placebo, a difference of 26 % as demonstrated by ridit analysis. In the FAS, the effective rate of FAHT cream was 36.28 % compared with an effective rate of 6.19 % of the placebo, a significant difference of 25 % as demonstrated by ridit analysis (Table S1 in the ESM).

Analysis of the PPS showed an integral therapeutic efficacy of 11.43 % for 4 weeks of treatment of FAHT cream for moderate and severe melasma compared with an integral therapeutic efficacy of 0.0 % for the placebo. This corresponds to a 21 % difference in clinical efficacy between the two groups as demonstrated by ridit analysis. In the FAS, the integral therapeutic efficacy of FAHT cream was 11.50 % compared with an integral therapeutic efficacy of 0.0 % for the placebo, a significant difference of 20 % as demonstrated by ridit analysis (Table S1, ESM).

3.2.3 Summary of Efficacy

A summary of efficacy is presented in Table S2 (see ESM). Taken together, these data demonstrate superior efficacy of FAHT cream compared with placebo for the treatment of moderate and severe melasma.

3.3 Safety

3.3.1 Dosing/Exposure Analysis

The exposure period of patients in the FAHT group was 53.96 ± 9.12 days, while that of subjects in the placebo group was 54.80 ± 7.65 days. There was no significant difference in duration of exposure between the two groups (Table 1).

3.3.2 Summary of Safety Evaluation

Of 113 subjects in the FAHT group, 35 (30.9 %) reported adverse reactions during the study period. One subject reported eye pain after mistakenly applying the cream improperly; this was considered to be unrelated to the FAHT treatment. The remaining 34 (of 113) patients had adverse reactions considered to be related to the FAHT treatment; therefore, the adverse rate was 30.1 %. In order of frequency, erythema, stabbing pain, peeling, telangiectasia, burning, red swelling, dry skin, itching, and darker pigmentation were sequentially found, although the severity of most occurrences was mild. Only two subjects discontinued the administration of FAHT cream because of severe adverse reactions. One subject experienced erythema (mild severity) and peeling (mild severity), and the other patient experienced redness and swelling (moderate severity) and telangiectasia (mild severity). Both subjects withdrew from the study over concerns of potential facial damage. The adverse symptoms gradually resolved after discontinuation of FAHT cream (Table 4). Skin atrophy, usually caused by corticosteroid hormone, was not observed because of the proper balance maintained by tretinoin. Of 113 subjects in the placebo group, six (5.3 %) reported adverse reactions during the study period. Two patients experienced anemia and one subject caught a cold; these reactions were unrelated to the study treatment. The remaining three subjects (of 113) reported burning, tautening, and itching, which were considered to be related to the study treatment; therefore, the adverse rate was 2.6 %. No severe or major adverse reactions were reported (Table 4).

Abnormal clinical laboratory tests were found in five subjects after treatment. Two cases were in the FAHT group: one subject with lower hemoglobin due to slight anemia, and one subject with urine leukocytes due to urinary infection. The remaining three cases were in the placebo group: one subject with increased leukocytes due to the upper respiratory tract infection, and two subjects with urine leukocytes due to urinary infection. After a summary evaluation of the above results, these abnormal results were determined to be unrelated to the treatment.

4 Discussion

The present multicenter study demonstrates that the clinical efficacy of FAHT is significantly superior to placebo for the treatment of melasma. Superiority was evaluated by clinical efficacy, instrumental measured efficacy, and integral therapeutic efficacy. Briefly, the efficacy of FAHT is significantly greater than placebo at 4 weeks of treatment, and the efficacy of FAHT is five to six times greater after 8 weeks of treatment than after 4 weeks of treatment. However, occasional or sporadic adverse reactions, including (in order of frequency) erythema, stabbing pain, and peeling, do not worsen or become severe with longer duration of treatment, and the incidence of adverse reactions does not increase with increasing duration of treatment.

In addition to associated factors of the endocrine system, feminine hormones, or oral contraceptive pills, ultraviolet (UV) light is considered a definite factor that often initiates or exacerbates melasma. Therefore, during our FAHT treatment, study subjects were advised to wear sunscreen when outdoors. UV exposure not only stimulates melanogenesis and melanocyte proliferation and migration but also induces the production of various cytokines, including α-melanocyte-stimulating hormone, and the production of adrenocorticotropic hormone from keratinocytes, which

Table 4 Characteristics and frequency of adverse reactions

Adverse reaction	Group (patients)	Cases [*n* (%)]	Severity level	
			Mild [*n* (%)]	Moderate [*n* (%)]
Erythema	FAHT (113)	21 (18.58)	18 (85.71)	3 (14.29)
	Placebo (113)	0 (0)		
Stabbing pain	FAHT (113)	9 (7.96)	8 (88.89)	1 (11.11)
	Placebo (113)	0 (0)		
Peeling	FAHT (113)	8 (7.08)	8 (100)	0 (0)
	Placebo (113)	0 (0)		
Telangiectasia	FAHT (113)	5 (4.42)	3 (60)	2 (40)
	Placebo (113)	0 (0)		
Burning	FAHT (113)	4 (3.54)	4 (100)	0 (0)
	Placebo (113)	1 (0.88)	1 (100)	0 (0)
Dry skin	FAHT (113)	3 (2.65)	3 (100)	0 (0)
	Placebo (113)	2 (1.77)	2 (100)	0 (0)
Itching	FAHT (113)	3 (2.65)	3 (100)	0 (0)
	Placebo (113)	1 (0.88)	1 (100)	0 (0)
Redness/swelling	FAHT (113)	2 (1.77)	1 (33.33)	2 (66.67)
	Placebo (113)	0 (0)		
Sensation of thin skin	FAHT (113)	1 (0.88)	1 (100)	0 (0)
	Placebo (113)	0 (0)		
Further pigmentation	FAHT (113)	1 (0.88)	1 (100)	0 (0)
	Placebo (113)	0 (0)		
Tautening	FAHT (113)	0 (0)		
	Placebo (113)	1 (0.88)	1 (100)	0 (0)
Eye pain	FAHT (113)	1 (0.88)	1 (100)	0 (0)
	Placebo (113)	0 (0)		
Cold	FAHT (113)	0 (0)		
	Placebo (113)	1 (0.88)	0 (0)	1 (100)
Anemia	FAHT (113)	0 (0)		
	Placebo (113)	2 (1.77)	1 (50)	1 (50)

FAHT fluocinolone acetonide, hydroquinone, and tretinoin cream

increase melanogenesis and melanocyte proliferation in a feedback loop [1].

Successful treatment for melasma is dependent upon inhibiting melanogenesis through a combination of sunscreen and other topical formulations as well as rooting out excessive melanin that exists in the epidermis and dermis. The latter goal is the most difficult. In 1999, Hill Dermaceuticals, Inc. (Sanford, FL, USA) submitted the NDA for the present triple formulation (0.01 % fluocinolone acetonide, 4 % hydroquinone, and 0.05 % tretinoin), claiming an additive or synergistic effect in the depigmentation of melasma based on the formulation of Kligman and Willis in 1975 [11, 12]. Hydroquinone is a depigmenting agent that works through inhibiting tyrosinase (blocking oxidation of tyrosine to dihydroxyphenylalanine, a precursor of melanin) and suppressing other melanocyte metabolic processes; however, such depigmentation from this process is reversible. Although the exact mechanism of tretinoin action on depigmentation is vaguely understood, the following pathways are possible:

1. inhibition of tyrosinase;
2. desquamation induced by keratolytic action;
3. interference with melanosome transfer in keratinocytes [9].

Currently, FAHT cream is considered first-line therapy for moderate and severe melasma, as previously reported [12, 13]. The results from the present study are consistent with previous reports, and we conclude that FAHT cream is highly efficacious for the treatment of moderate and severe melasma.

The Melasma Area and Severity Index (MASI) is widely employed to evaluate the efficacy of melasma; however, this measurement method is highly subjective and often complicated. Two indices for measuring the extent of changes in darkness and area of the damaged skin were

used for scoring in the present study, by virtue of the diagnostic and treatment criteria of melasma [14] recommended by the Pigmentation Disease Division, Committee of Skin and Venereal Disease, China Society of Integrated Traditional Chinese and Western Medicine. Furthermore, to avoid subjective bias, instrumental measurements were used to objectively determine the extent of freckle fading. Taylor et al. reported from the US that Tri-Luma®, used with an identical dosage as FAHT cream, exhibited an effective rate of up to 77 % for the treatment of moderate and severe melasma [11]. In this study, the clinical efficacy of FAHT cream was 68.57 %, which is close to the 64.2 % in a study conducted mostly on Asian patients with moderate and severe melasma reported by Chan et al. [15]. The efficacy differences may be attributed, on the one hand, to varying inclusion criteria and methods of evaluation and, on the other hand, to different races.

Compared with previous reports, the rate of adverse reactions in this study is lower, although the incidence rates are similar. Most of these adverse reactions could be predicted because they were triggered by the active ingredients, hydroquinone and tretinoin, of the FAHT cream [16]; however, most of the adverse reactions were very mild and were resolved after discontinuation of administration.

5 Conclusion

This study demonstrates that FAHT cream is highly efficacious, and has a good margin of safety and tolerance for the treatment of moderate and severe melasma in the Chinese population.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee as well as the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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