A Histological Examination for Skin Atrophy After 6 Months of Treatment With Fluocinolone Acetonide 0.01%, Hydroquinone 4%, and Tretinoin 0.05% Cream

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Abstract: Melasma is a common disorder affecting a significant percentage of the population, particularly those with skin of color. Therapy with hydroquinone, a depigmenting agent, as a single agent or in combination with other agents has been used with variable success. A triple-combination (TC) cream combining hydroquinone 4% with tretinoin 0.05% and fluocinolone acetonide 0.01% was developed for the treatment of melasma. We studied the use of TC cream for 24 weeks and had tissue samples for all time points in 62 patients with moderate to severe melasma. The atrophogenic potential of TC cream was evaluated through serial histopathologic examination of skin biopsies. No statistically significant histopathologic signs of atrophy of the epidermis or dermis were noted at any time point throughout the study. There was a marked reduction in epidermal melanin in treated subjects; however, we did not observe any significant difference in baseline and treated samples in the amount of perivascular inflammatory infiltrate, dermal mucin, keratinocyte and melanocyte atypia, or mast cells, consistent with findings of previous studies where topical retinoids were used. An increase in the mean number of blood vessels per square millimeter of tissue was observed in 2 study cohorts between baseline and week 24. These results suggest that the risk of skin atrophy with 24-week use of TC cream for the treatment of melasma is very low.

Key Words: melasma, tretinoin, hydroquinone, fluocinolone acetonide, skin atrophy

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INTRODUCTION

Melasma is a common disorder that affects a significant percentage of the population, particularly those of darker color. Hydroquinone, a depigmenting agent, as a single agent or in combination with other agents has been used with variable success.[1–5] A triple-combination (TC) cream combining hydroquinone 4% with tretinoin 0.05% and fluocinolone acetonide 0.01% was developed for the treatment of melasma.[6,7] Fluocinolone acetonide 0.01% is a low-potency, class 6, fluorinated corticosteroid. It is often confused with more potent steroids, resulting in hesitancy on the part of dermatologists for long-term use on the face.[4,8] Previous studies have demonstrated the efficacy and safety of TC cream for short-term and intermittent long-term treatment of melasma.[6,9,10] Despite the low clinical incidence of atrophy reported in these trials, concerns about atrophy linger; therefore, this study was conducted to assess atrophogenic potential of TC cream with the extended (24 weeks) use through histopathologic examination of skin biopsies at baseline, 12 weeks, and 24 weeks. The atrophogenic potential, with 24-week treatment of melasma, of TC cream was evaluated through histopathologic examination of skin biopsies.

METHODS

The inclusion criteria included patients aged 18–65 years with moderate to severe melasma. There were no sex or racial exclusions. Pregnancy and purely dermal melasma were exclusion criteria. TC cream was applied once daily for a minimum of 12 weeks and continued until an investigator assessment of clear or almost clear was attained. Upon achieving clear or almost clear (at week 12 or beyond), patients entered the maintenance phase, which consisted of applying TC cream only twice a week. For maintenance patients whose condition relapsed, at the discretion of the investigator, a daily dosing schedule was resumed. If the assessment of clear or almost clear was not obtained, the patient continued with the once-daily regimen for the entire study period (24 weeks).

Skin biopsies (2-mm punch biopsy) were performed at baseline (one in an involved area and one in a nearby noninvolved area), at week 12, and at week 24 (post-baseline biopsies of involved skin only). All biopsy specimens were taken from the same site (cheek) in all subjects to avoid variability due to location. All samples were mailed to the Skin Pathology Laboratory at Boston University School of Medicine. Biopsy specimens were coded by a laboratory number only, so that all evaluators were unaware of the subject number and type of skin (uninvolved or involved). All biopsy

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specimens were examined as a group at the end of the study to minimize investigator and processing variability. The tissue samples were fixed in 10% neutral buffered formalin, and samples were processed for routine paraffin sectioning. Four-micron sections were stained with hematoxylin and eosin, Fontana-Masson, Verhoeff-Van Gieson, colloidal iron, and chloroesterase. Hematoxylin and eosin stains were reviewed in detail to determine the general histopathologic features such as stratum corneum compaction, number of granular cell layers, keratinocytic and melanocytic atypia, and perivascular inflammation. The latter 3 items were graded as none, scant (minimal to mild), moderate, or abundant (severe). The grading was based on the number of inflammatory cells surrounding the average vascular cross section and degree of atypia. Dermal mucin stained by colloidal iron was graded as 0–3 (3 being the strongest intensity). Chloroesterase was used to stain mast cells. Fontana-Masson was used for the determination of epidermal melanin, and Verhoeff-Van Gieson staining was done to evaluate elastic tissue in the dermis by computerized image analysis as described earlier.[11] In addition, immunostaining with MART-1 (predilute; Ventana Medical Systems, Inc, Tucson, AZ) and

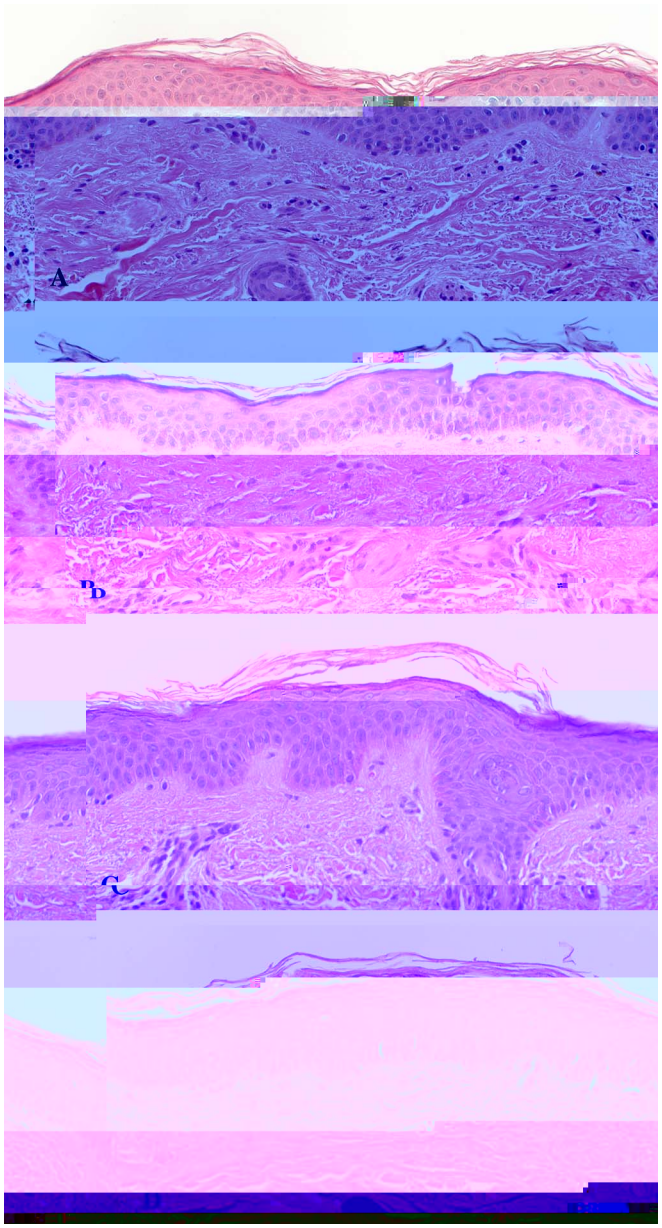

FIGURE 1. Epidermal thickness. Histologic images of representative epidermal layers at baseline (A: uninvolved vs. B: involved) and in involved areas after 12 weeks (C) and 24 (D) weeks of treatment. All images are from the same patient.

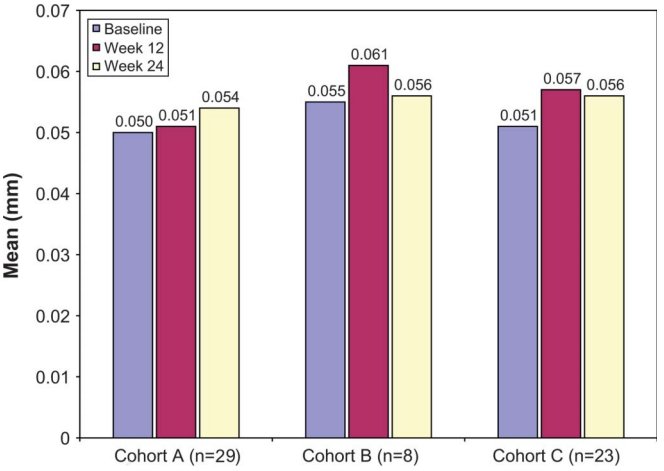

FIGURE 2. Mean epidermal thickness. Histologic images and mean epidermal thickness (millimeters) measurements at baseline, week 12, and week 24 in treated skin indicate that there was no evidence of epidermal atrophy (no statistically significant differences in epidermal thickness) at any time point in the study. Mixed-effects regression was used to compare measurements of epidermal thickness of involved areas at weeks 12 and 24 with involved areas at baseline. Statistical analyses were not conducted on cohort B due to small sample size.

vessels indicated that there was an increase in the mean number of blood vessels (per square millimeter) after 24 weeks of treatment in treated skin (Fig. 5), but no mean increase at 12 weeks. In cohort A, the mean number of blood vessels significantly increased from 34.5 to 50.1 by week 24 (P = 0.007). In cohort B, the mean number of blood vessels increased from 24.1 to 49.3 by week 24; however, statistical analysis in this cohort was not done due to the small sample size (n = 8). In cohort C, the mean number of blood vessels significantly increased from 31.6 to 43.8 by week 24 (P = 0.02). There was no significant change over time for any cohort in epidermal melanocytes in the samples of treated skin at 12 and 24 weeks of treatment.[4]

As expected, there was a significant reduction in epidermal melanin in treated samples in comparison to the baseline values at week 12 for cohort C (P = 0.027) and at week 24 for cohort A (P = 0.029). No significant difference was observed in the elastic tissue contents between treated (12 and 24 weeks) and baseline skin samples for any cohort. Similarly, there was no difference between the mast cell counts of treated samples at 12 or 24 weeks in comparison to the baseline values for any cohort. Dermal mucin was comparable in treated samples in both groups and the untreated sites. There was no keratinocytic or melanocytic atypia in any of the skin samples (baseline or untreated). The number of granular cell layers increased in treated subjects in cohorts A and C at 12 and 24 weeks, which was statistically significant in comparison to the baseline involved skin. Similarly, the stratum corneum changed from basket weave to compact type in cohorts A and C at both 12 and 24 weeks in comparison to the baseline involved skin, which was also statistically significant. A slight increase in perivascular lymphocytic infiltrate was seen in all cohorts of patients at 12 and 24 weeks in comparison to baseline involved skin; however, this was not statistically significant.

Cohort A exhibited statistically significant (P = 0.008) differences in the distribution among stain grades between week 24 and baseline in the expression of procollagen I, whereas cohort C exhibited statistically significant ($P < 0.001$) differences in the distribution among stain grades between week 24 and baseline in the expression of collagen III. Although statistically significant differences were noted between baseline and week 24, statistical tests for symmetry and relationships revealed that the data for collagen III and

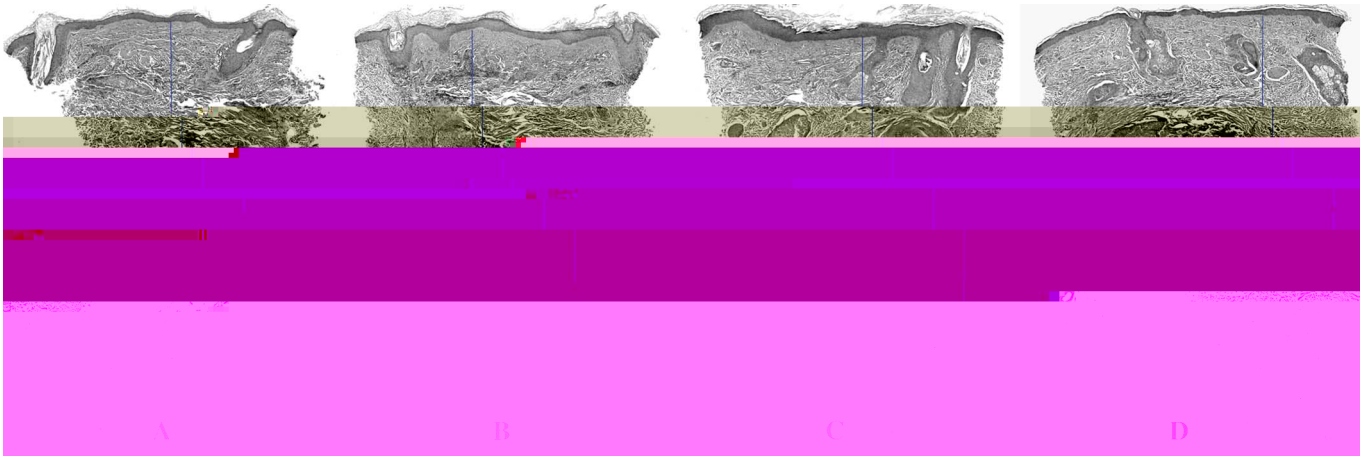

FIGURE 3. Dermal thickness. Histologic images of dermal layers at baseline (A: uninvolved vs. B: involved) and in involved areas after 12 weeks (C) and 24 (D) weeks of treatment. The blue line depicts the length of the dermal layer in each image. All images are from the same patient.

procollagen I were too variable to draw any conclusions even with statistical significance (tests for symmetry and relationships in both cohorts were not statistically significant).

DISCUSSION

Melasma, a macular hyperpigmentation disorder, remains a challenge in terms of effective treatment. Hydroquinone 4%, tretinoin 0.05%, and fluocinolone acetonide 0.01% have been formulated as a topical TC cream, which has demonstrated beneficial results to patients with this disorder. Because of the potential atrophogenic effect of the fluocinolone acetonide component of this product, there are safety concerns. Our aim was to evaluate the effects of TC over 24 weeks of treatment in a multicenter study.

This study is the first to assess the atrophogenic potential of a TC cream in the treatment of melasma using histologic evaluations of skin biopsies. The results clearly demonstrate that there is no histologic evidence of epidermal or dermal atrophy in patients treated for up to 24 weeks with TC. There was a statistically significant decrease in epidermal melanin content at week 12 for cohort C ($P = 0.027$) and at week 24 for cohort A ($P = 0.029$), but the reduction was not evaluated statistically in cohort B, in which patients had daily treatment for 12 weeks followed by 12 weeks of only twice-weekly

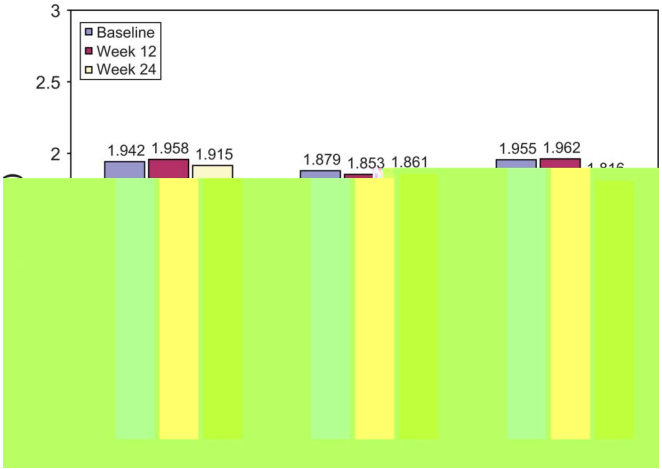

FIGURE 4. Mean dermal thickness (millimeters). Safety population. Histologic images and mean dermal thickness measurements from treated skin indicate that there was no evidence of dermal atrophy (no statistically significant differences in dermal thickness) at any time point in the study. Mixed-effects regression was used to compare measurements of dermal thickness of involved areas at weeks 12 and 24 with involved areas at baseline. Statistical analyses were not conducted on cohort B due to small sample size.

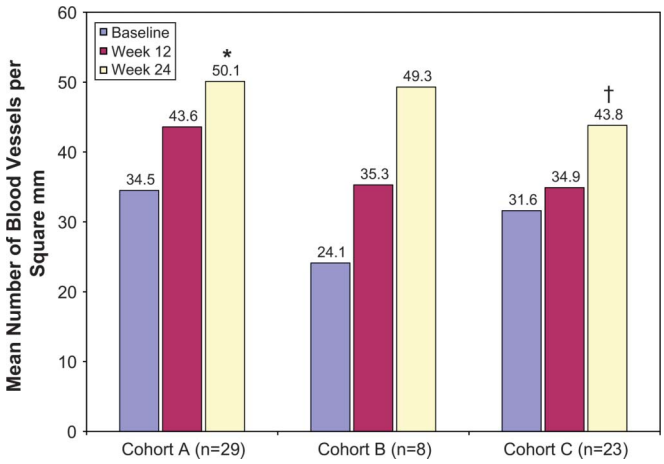

FIGURE 5. Histopathology analysis of blood vessels. Safety population. *$P < 0.001$. †$P = 0.005$. *P* values represent comparison of values of week 24 to baseline (involved areas), mixed-effects regression; *P* value for cohort B was not calculated due to small sample size. There was a significant increase in the mean number of blood vessels (per square millimeter) in cohorts A and C after 24 weeks of treatment in comparison to baseline in involved areas.

maintenance treatment, because of small sample size. Statistically significant stratum corneum compaction and increase in granular layers were seen in cohort A ($P < 0.001$) and cohort C ($P = 0.006$), which were in keeping with well-established retinoid effects on skin.[11–13] Again, these changes were not evaluated statistically in cohort B. Our study did not demonstrate any significant difference in baseline and treated samples in the amount of perivascular inflammatory infiltrate, dermal mucin, keratinocyte and melanocyte atypia, or mast cells. These findings are also consistent with previous studies where topical retinoids were used.[11–13] Although some reports suggest that increased collagen III and procollagen I are markers of collagen synthesis with use of topical retinoids,[14,15] we did not observe any such finding. Our findings show that, for daily use of TC over 24 weeks (cohort A), collagen III remained unchanged, whereas procollagen I tended to decrease. However, tests for symmetry and relationships revealed that the data for collagen III and procollagen I were too variable to draw conclusions even when statistically significant differences were noted. Taken in concert with the fact that reliable and objective measurements for dermal and epidermal atrophy did not demonstrate any statistically significant differences over time, it is not possible to deem the collagen III and procollagen I results from this study as predictors or markers of atrophy.

It is interesting to point out that there was a significant increase in the mean number of blood vessels after 24 weeks of treatment in cohort A ($P = 0.007$) and cohort C ($P = 0.020$). The significance of this is not clear but may correlate with clinical erythema seen after topical retinoid use.[16] This increase could also be secondary to steroid use. A recent study by Kim et al[17] evaluated the vascular characteristics in melasma lesions by evaluating skin samples from 50 Korean women with melasma. They found a significant relationship between the number of vessels and melasma lesions compared with nonlesional adjacent skin, and the expression of vascular endothelial growth factor was significantly increased in melasma-affected skin. Another report by Rendon et al[18] describes a series of patients in which they were able to detect melasma with underlying telangiectasias that were not associated with treatment using topical corticosteroids. Additional studies are needed to clarify whether telangiectasias appear as a result of melasma treatment or simply become more visible when they are no longer hidden behind hyperpigmented lesions.

CONCLUSIONS

In conclusion, there was no histologic evidence of epidermal or dermal atrophy for any patient at any time point during the 24-week study after applying TC cream. There was a marked reduction in epidermal melanin in treated subjects. These results indicate that the risk of skin atrophy with 24-week use of TC cream for the treatment of melasma seems to be very low.

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