ORIGINAL RESEARCH ARTICLE

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Long-Term Efficacy and Safety of Tretinoin Emollient Cream 0.05% in the Treatment of Photodamaged Facial Skin

A Two-Year, Randomized, Placebo-Controlled Trial

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Abstract

Background: Long-term (>1 year) placebo-controlled studies of tretinoin in the treatment of photodamaged skin have not been conducted. Recently, we conducted a 2-year placebo-controlled study of tretinoin emollient cream 0.05%, including histopathologic assessment of safety and analysis of markers of collagen deposition.

Objective: The objective of the study was to determine the long-term safety and efficacy of tretinoin emollient cream 0.05% in the treatment of moderate to severe facial photodamage.

Methods: A total of 204 subjects were treated with tretinoin or placebo (vehicle emollient cream) applied to the entire face once a day for up to 2 years. Clinical and histologic effects were assessed at regularly scheduled clinic visits.

Results: Treatment with tretinoin resulted in significantly greater improvement relative to placebo in clinical signs of photodamage (fine and coarse wrinkling, mottled hyperpigmentation, lentigines, and sallowness), overall photodamage severity, and investigator's global assessment of clinical response ($p < 0.05$). Histologic evaluation showed no increase in keratinocytic or melanocytic atypia, dermal elastosis, or untoward effects on

stratum corneum following treatment with tretinoin compared with placebo. Immunohistochemistry studies, conducted at three study centers, showed a significant increase relative to placebo in facial procollagen 1C terminal, a marker for procollagen synthesis, at month 12 ($p = 0.0074$).

Conclusion: Long-term treatment with tretinoin emollient cream 0.05% is safe and effective in subjects with moderate to severe facial photodamage.

Photodamage to the skin, caused by repeated exposure to ultraviolet radiation from sunlight, is characterized by clinical signs such as skin roughness, fine and coarse wrinkling, mottled hyperpigmentation, lentigines, and sallowness. Histopathologic studies have revealed alterations in the dermal extracellular matrix of photodamaged skin characterized by a disorganization and degradation of the collagenous meshwork and an accumulation of elastotic material comprised primarily of elastin fibers.[1-4] This damage to the extracellular matrix, which normally provides the mechanical support for the epidermis, is believed to be responsible in large part for the wrinkled appearance of photodamaged skin.

Numerous studies, including large-scale multicenter trials, have established that topical tretinoin is safe and effective in reducing fine facial wrinkling, mottled hyperpigmentation, and skin roughness in subjects with photodamaged skin.[5-10] Placebo-controlled studies have shown that topical tretinoin therapy for 3–6 months causes improvement in a number of histologic parameters, without untoward dermal effects.[5,11] Bhawan et al.[11] reported the histologic effects of topical tretinoin therapy on photodamaged skin in 533 patients participating in two 24-week multicenter, double-blind, randomized, placebo-controlled trials.[9,10] Compared with placebo, 24 weeks of therapy with tretinoin emollient cream (0.001%, 0.1%, 0.05%) resulted in dose-dependent epidermal changes including increases in epidermal thickness, increased granular layer thickness, decreased melanin content, and stratum corneum compaction. No obvious adverse dermal changes were detected in these subjects. In an extension study of the two controlled clinical trials, subjects who completed 24 weeks of therapy with tretinoin emollient cream 0.01% or 0.05% were continued on the same regimen for an additional 24 weeks.[12,13] Histologic data from 298 patients showed that, with the exception of decreased melanin content, the improvement in epidermal parameters seen at 24 weeks returned to baseline levels after 48 weeks of therapy, despite a persistence of clinical benefits in skin roughness and fine wrinkling.[13] There was no evidence of increased keratinocytic atypia or melanocytic atypia compared with baseline specimens in subjects treated with tretinoin for up to 48 weeks. Further, no untoward effects on keratinocytes or melanocytes were observed in 27 subjects who received tretinoin therapy for up to 4 years.[14] The histologic data from these studies suggest that prolonged topical tretinoin therapy causes no safety concerns.[13,14]

We report the results of a prospective, 2-year, double-blind, placebo-controlled study of tretinoin emollient cream 0.05%, including a histopathologic assessment of safety and an analysis of markers of procollagen formation. Our data confirm the long-term efficacy and safety of tretinoin in subjects with moderate to severe photodamaged facial skin.

Methods

This was a prospective, 2-year, double-blind, randomized, placebo-controlled, multicenter study. Subjects who satisfied all the entry criteria were randomized to treatment with tretinoin emollient cream 0.05% or placebo (vehicle emollient cream). Subjects were instructed to apply a pea-size amount (approximately 0.25g) of study cream to the entire face once a day at night after washing the face with a skin cleanser. A moisturizer with sunscreen was to be applied daily, and an additional sunscreen (SPF 30) was to be applied prior to extended periods of exposure to ultraviolet light. If excess irritation occurred, less frequent applications could be made, or the study cream could be discontinued temporarily as directed by the investigator. Short-term therapy (i.e. ≤5 days) with a topical corticosteroid was also permitted in the event of excessive skin irritation. No topical medication except study cream was to be applied to the face during the study. The use of cosmetics was to be minimized. Neither study cream nor any other approved emollients were to be applied to the face for 24 hours prior to study visits. Cosmetics were not to be applied on the day of study visits. Duration of treatment was 24 months.

Efficacy and safety evaluations were performed during clinic visits at months 1, 2, 4, 6, 9, 12, 15, 18, 21, and 24. The investigator's clinical assessments of photodamage (tactile roughness, fine wrinkling, coarse wrinkling, mottled hyperpigmentation, lentigines, yellowing/sallowness), cutaneous irritation (erythema, peeling, dryness, itching, burning/stinging), and the subject's self-assessments (skin texture, overall appearance and feel of skin, skin color, skin pores, small wrinkles, skin tightness) were performed at each visit. The investigator's global assessment of clinical response was determined at months 6, 12, 18, and 24. Photographs and skin biopsies (periorbital) were taken at baseline,

12 months, and 24 months. Adverse events were recorded at all study visits.

Severity ratings for clinical assessments of photodamage and cutaneous irritation were graded on a ten-point scale, categorized into four levels as follows: 0 = none, 1–3 = mild, 4–6 = moderate, and 7–9 = severe. The global assessment was rated as much improved, improved, slightly improved, no change, or worse. Punch biopsy specimens (2mm) were taken from the left periorbital (crow's foot) area under local anesthesia. Biopsy specimens from three study centers were shipped immediately in dry ice to the University of Michigan (Ann Arbor, MI) for immunohistologic evaluation of procollagen 1C terminal (PIC) and procollagen 1 (SP1) as indicators of procollagen synthesis. Control biopsy specimens were obtained from a photo-protected area (buttocks) at these three centers. The other study centers sent periorbital biopsy specimens to a central laboratory for standard hematoxylin and eosin staining. Three independent dermatopathologists blinded to study treatment rated histology variables (keratinocytic dysplasia, melanocytic dysplasia, dermal elastosis, and stratum corneum compaction).

The study protocol was approved by the Institutional Review Board of each participating study site. The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki. Prior to enrollment, the nature and potential risks of the study were explained to all potential participants, and written informed consent was obtained from all subjects.

Study Population

A total of 204 subjects were enrolled and randomized into the study. Subjects were recruited from 17 qualified clinical sites. Random assignment to study drug was evenly distributed at each site.

Inclusion Criteria

Eligible subjects included men and non-pregnant, non-nursing women in good health, between the ages of 30 and 75 years, with lightly pigmented skin (Fitzpatrick skin type I-III). Subjects were required to have moderate to severe photodamaged facial skin (overall severity grade of 6–9 at baseline), with a clinical grade of 6−8 at baseline for fine wrinkles. Subjects were required to discontinue all topical retinoids and products containing α-hydroxy acids 30 days prior to starting treatment, systemic retinoids (other than normal recommended daily allowance of vitamin A) and skin bleaching agents at least 3 months before starting treatment. In addition, all other topical medications and emollients to the face had to be discontinued at least 24 hours before pre-study evaluations. No cosmetics could be applied on the day of pre-study evaluation.

Exclusion Criteria

Subjects were excluded from the study if they had a known history of precancerous lesions or more than three precancerous lesions in the past year, a history within the past year of basal cell or squamous cell carcinoma on the face, or a past history of malignant melanoma at any site; had actinic keratosis (face) at enrollment; received therapy within the past year for photodamaged or aging facial skin; had any skin condition that could require concurrent therapy or confound the evaluation of drug safety or efficacy; had a history of psychotic or affective disorders, keloid formation, or hypersensitivity to any of the formulation components of the study medications; had excessive facial hair; required electrolysis, waxing, or depilatories on the face during the conduct of the study; were using any known photosensitizing agents; or had received any experimental drug or used any experimental device within 30 days prior to initiation of study therapy.

Treatment Groups

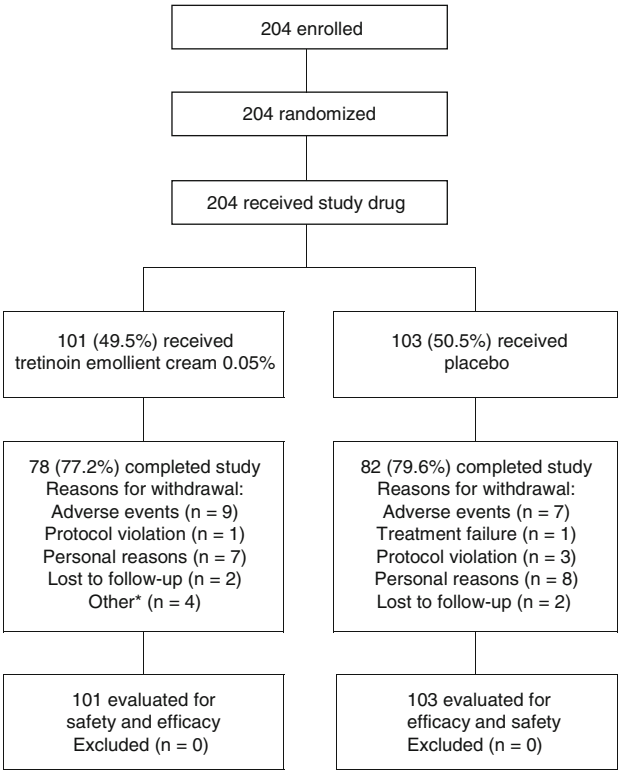
Computer-generated randomization lists, using a pre-specified block size for each study site, were used to package and label the double-blinded treatment. Subjects who satisfied all the entry criteria were randomized in a 1 : 1 ratio to tretinoin emollient cream 0.05% or placebo according to the randomization plan. Placebo was formulated as a color-matched emollient cream containing vehicle components only (i.e. all constituents of the marketed product except the active ingredient). The investigators, other study personnel, and subjects were blinded to the study treatment. The blind was to be broken only in the event that emergency treatment required knowledge of the drug administered. In an emergency situation, the metallic surface of the blinded area of the label could be scratched off.

Criteria for Evaluation

The evaluation of clinical signs of photodamage, the overall severity of photodamage, the investigator's global assessment of treatment response, and the subject's self-assessment provided a profile of efficacy. Serially obtained biopsies were utilized to document changes in histologic patterns during long-term administration and provide a safety comparison between active product and placebo. Other safety parameters included the incidence of adverse events and severity grading of signs and symptoms of cutaneous irritation.

Immunohistology

Skin cryo-sections (7 microns) were air dried then fixed in cold acetone for 10 minutes. Sections were preincubated with normal



*Other reasons for premature withdrawal from the study included exclusionary surgery/procedure (2 subjects), investigator request (1 subject), and subject request (1 subject).

Fig. 1. Flow chart of subject participation during the randomized controlled trial.

horse serum (1.5%) for 30 minutes. Subsequently, the slides were incubated for 1 hour at room temperature with mouse monoclonal antibodies against the amino terminal extension peptide of procollagen type I (SP1, 1.9 μg/mL, Developmental Studies Hybridoma Bank) maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University of Medicine (Baltimore, MD) and the Department of Biology, University of Iowa (Iowa City, IA), and the carboxy terminal peptide of procollagen type I (PIC, 0.29 mg/mL, TaKaRa Biomedical Madison, WI), or mouse IgG isotype control (Sigma Chemical Co., St Louis, MO). Then, sections were incubated with a biotinylated horse anti-mouse antibody (1 **:** 200) for 30 minutes. Sections were then incubated with avidin biotin peroxidase complex (1 **:** 50, Vector Laboratories Inc., Burlingame, CA) for 30 minutes. 3-Amino-9-ethyl carbazole (Sigma Chemical Co.) was used as chromogen. Between steps, the slides were rinsed for 10 minutes in phosphatase buffered saline with 0.1% Triton-X-100. All sections were lightly counterstained with hematoxylin.

Statistical Analyses

The planned sample size for this study was based on estimates of treatment differences that would be clinically meaningful. Using a two-group t-test with a two-sided significance level of 0.05, inclusion of 75 subjects in each treatment group was determined to have 80% power to detect a difference in means of 0.5 units, assuming a standard deviation of 1 and a dropout rate of 15%. The same sample size was required to detect a 1-unit difference in means, assuming a standard deviation of 2. The sample size also accommodated detection of differences in proportions of 0.25–0.30 with 80% power.

The primary population for efficacy evaluation was the intention-to-treat population, which comprised all randomized subjects who had at least a baseline efficacy evaluation. All randomized subjects who received any study medication were evaluated for safety. The primary efficacy variables were the clinical signs of fine wrinkling, mottled hyperpigmentation, and tactile roughness. The primary efficacy endpoint was the change from baseline to month 24 in severity ratings for each of these three variables. The Cochran-Mantel-Haenszel (CMH) row-mean score test statistic, stratified by investigator, was used to compare treatment groups (tretinoin emollient cream 0.05% vs placebo) for the change in severity ratings from baseline to month 24. The sequentially rejective Bonferroni-Holms procedure was used to maintain a family-wise error rate of 5% based on testing of the treatment differences for fine wrinkling, mottled hyperpigmentation, and tactile roughness. The CMH row-mean score test statistic, stratified by investigator, was used for secondary efficacy analyses, including treatment comparisons for the change from baseline to each subsequent visit in severity of individual clinical signs, overall severity of photodamage, and subject self-assessment ratings. This test was also used to evaluate treatment differences in the investigator's global evaluation ratings at months 6, 12, 18, and 24, and change from baseline at months 12 and 24 in severity of each of the histology variables. A baseline-adjusted analysis of covariance was used to compare treatment groups for change from baseline at months 12 and 24 in immunohistologic variables (PIC and SP1).

Results

Of the 204 subjects randomized into the study, 101 received tretinoin emollient cream 0.05% and 103 received placebo. All subjects were included in the intention-to-treat analysis which compared baseline with results at endpoint (last observation carried forward). A flow chart of subject participation during the study is provided in figure 1.

Table I. Demographics, skin type, and disease severity[a]

Variable	Tretinoin emollient cream 0.05% (n = 101)	Placebo (n = 103)
Age (years)		
Mean ± SD	63.2 ± 7.92	62.4 ± 8.13
Range	(40–76)	(43–75)
Gender, n (%)		
Male	10 (9.9%)	11 (10.7%)
Female	91 (90.1%)	92 (89.3%)
Race, n (%)		
White	97 (96.0%)	97 (94.2%)
Other	4 (4.0%)	6 (5.8%)
Overall photodamage severity, n (%)		
9 (severe)	0	2 (1.9%)
8 (severe)	14 (13.9%)	10 (9.7%)
7 (severe)	39 (38.6%)	41 (39.8%)
6 (moderate)	48 (47.5%)	50 (48.5%)
Fitzpatrick skin type, n (%)		
I	13 (12.9%)	9 (8.7%)
II	41 (40.6%)	47 (45.6%)
III	47 (46.5%)	47 (45.6%)

a Percentages may not add up to 100% because of rounding.

The treatment groups were balanced at baseline with respect to severity of clinical signs of photodamage and cutaneous irritation scores. Approximately 60% of subjects had severe ratings (grades 7–9) at baseline for fine wrinkling and approximately 50% for coarse wrinkling. Severe ratings at baseline for sallowness (approximately 30%), mottled hyperpigmentation (approximately 20%), lentigines (approximately 20%), and tactile roughness (<10%) were less common. The majority of subjects (>85%) exhibited no peeling, itching, or burning/stinging at baseline. Approximately 50% of subjects had cutaneous erythema and dryness. The intensity of erythema and dryness was mild in approximately 40% of subjects. Subject demographics, skin type, and overall disease severity at baseline are summarized in table I.

Clinical Response

Treatment with tretinoin emollient cream 0.05% for 24 months resulted in significant improvement relative to placebo in fine wrinkling and mottled hyperpigmentation (p = 0.002; figure 2). Change from baseline to month 24 in tactile roughness was not significantly different between treatment groups (p = 0.645). Other clinical signs of photodamage (secondary efficacy variables) showed significant improvement after 24 months of treatment with

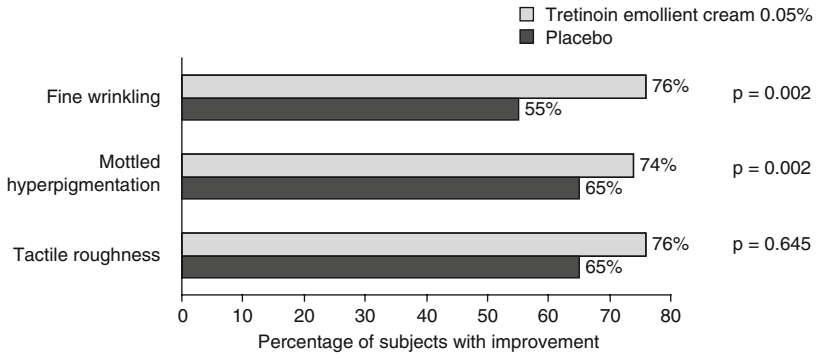


Fig. 2. Percentage of subjects with improvement from baseline to month 24 in primary efficacy variables (fine wrinkling, mottled hyperpigmentation, tactile roughness) as assessed by the investigator. Improvement was defined as change from baseline to month 24 in severity ratings of –1 to –9.

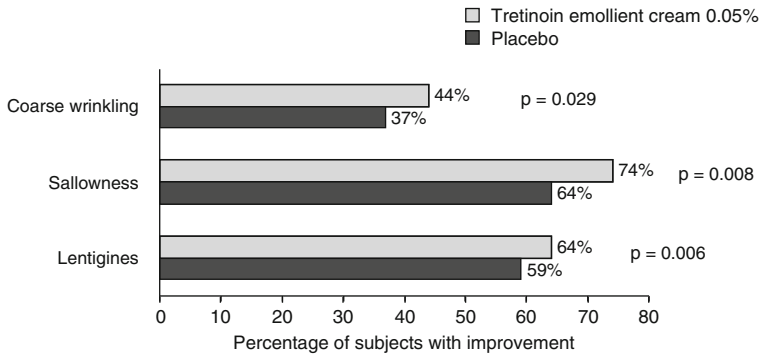


Fig. 3. Percentage of subjects with improvement from baseline to month 24 in coarse wrinkling, sallowness, and lentigines as assessed by the investigator. Improvement was defined as change from baseline to month 24 in severity ratings of –1 to –9.

tretinoin relative to placebo ($p < 0.03$; figure 3). Significant improvement was observed as early as 1 month for coarse wrinkling, 2 months for fine wrinkling, and 4 months for mottled hyperpigmentation, sallowness, and lentigines ($p < 0.05$). The overall severity of photodamage at month 24 was significantly reduced following treatment with tretinoin relative to placebo ($p = 0.006$). Improvement in overall severity with tretinoin compared with placebo reached significance by month 4 and continued through the remainder of the 24-month treatment period ($p < 0.05$). Figure 4 is a case sample of a subject treated with tretinoin emollient cream (baseline and month 24). The investigator's global assessment of clinical response at month 24 also showed a significant improvement with tretinoin relative to placebo ($p = 0.002$). The difference between treatment groups was significant in favor of tretinoin at all clinical assessments (months 6, 12, 18, and 24) [$p < 0.02$]. The analysis of individual subject self-assessment variables showed a significant difference between treatment groups in favor of tretinoin for fine wrinkles ($p = 0.026$). Treatment-group comparisons of the other self-assessment variables were not significantly different; nor were scores for overall appearance of the skin ($p = 0.727$).

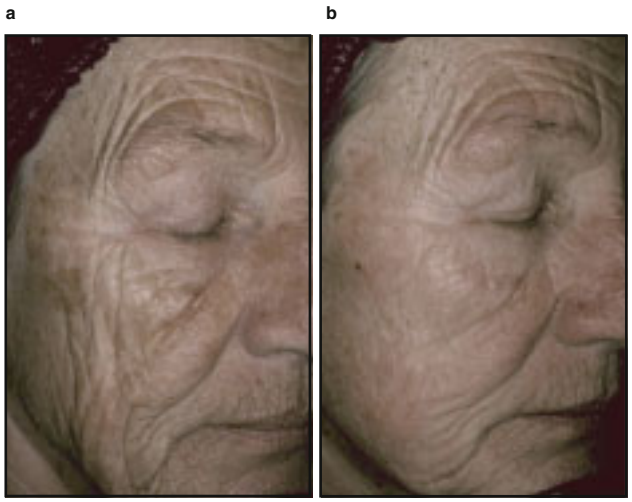


Fig. 4. Case example of a subject treated with tretinoin emollient cream 0.05%. (**a**) Baseline. (**b**) After 24 months of treatment.

Safety

Histologic pattern did not change, or changed only minimally, for the majority of subjects over the 2-year treatment period. All three independent dermatopathologists rated the majority of subjects (≥85%) in both treatment groups as having no change or a severity rating change of ±1 from baseline in each of the individual histology parameters (keratinocytic and melanocytic dysplasia, dermal elastosis, and stratum corneum) at months 12 and 24. There were no significant differences between treatment groups in the change from baseline to month 24 for any of the histology variables ($p > 0.05$). A summary of the distribution of subjects by change from baseline to month 24 in severity score for each of the histology variables averaged across the three independent raters is provided in table II.

The immunohistology indicators (PIC and SP1) in facial biopsies and the face-to-buttocks ratio of PIC and SP1 tended to show an increase from baseline in the tretinoin group relative to the placebo group at the post-treatment assessments (figure 5a and figure 5b). The treatment-group comparison for change from baseline to month 12 in PIC facial measurements was significant in favor of tretinoin ($p = 0.0074$). These data suggest there may be an increase in procollagen formation following long-term treatment with tretinoin emollient cream.

The proportion of subjects who experienced an increase in cutaneous irritation following treatment was higher in the tretinoin group than in the placebo group. Mean cutaneous irritation severity scores in both treatment groups increased after the start of treatment and peaked during the first 2 months. The severity of this adverse effect was judged by the investigator to be mild (scores of 1–3) for the majority of affected patients throughout the treatment period. After 2 years of therapy with tretinoin emollient cream, the

Table II. Number and percentage of subjects by change in histology ratings from baseline to month 24: average of three independent raters[a]

Change in rating[b]	Keratinocytic dysplasia		Melanocytic dysplasia		Dermal elastosis		Stratum corneum	
	tretinoin (n = 101)	placebo (n = 103)	tretinoin (n = 101)	placebo (n = 103)	tretinoin (n = 101)	placebo (n = 103)	tretinoin (n = 101)	placebo (n = 103)
>+1	0	0	0	0	4 (6%)	3 (4%)	1 (1%)	0
+1	13 (19%)	13 (19%)	8 (12%)	8 (12%)	16 (23%)	18 (26%)	7 (10%)	5 (7%)
0	49 (71%)	47 (68%)	51 (74%)	50 (72%)	40 (58%)	40 (57%)	51 (74%)	53 (77%)
−1	7 (10%)	9 (13%)	9 (13%)	10 (14%)	8 (12%)	8 (11%)	10 (14%)	10 (14%)
<−1	0	0	0	0	1 (1%)	1 (1%)	0	1 (1%)
n	69	69	69	69	69	70	69	69

a Numbers of subjects in each severity rating category may not add up to n because of rounding. Percentages are based on n.

b + = worsening; 0 = no change; − = improvement.

n = average number of subjects evaluated by each independent rater.

majority of subjects exhibited either no change or improvement from baseline on several features of irritation: erythema 73% (74/101) of subjects, peeling 67% (68/101), itching 82% (83/101), burning/stinging 84% (85/101), and dryness 74% (75/101).

The overall incidence of adverse events was similar in the tretinoin (83%) and placebo (82%) treatment groups (table III). The most common treatment-related adverse events were those affecting the skin, which occurred in higher incidence in the tretinoin group (30%; 31/101) than in the placebo group (9%; 9/103). Most adverse events were judged by the investigator to be mild or moderate in severity. The proportion of subjects who withdrew from the study as a result of adverse events was small and similar in the two treatment groups: 9% (9/101) in the tretinoin emollient cream group and 7% (7/103) in the placebo group.

Discussion

The results of this study demonstrated that tretinoin emollient cream 0.05% applied topically to the face once a day is safe and effective for up to 2 years in subjects with moderate to severe photodamaged skin. Treatment with tretinoin resulted in significant improvements relative to placebo in fine and coarse wrinkling, mottled hyperpigmentation, sallowness, and lentigines, although the improvement in tactile roughness was not significant compared with placebo. Significant improvement was observed by 1 month for coarse wrinkling, 2 months for fine wrinkling, and 4 months for mottled hyperpigmentation, sallowness, and lentigines. The data for individual clinical signs of photodamage were supported by significant improvement in overall severity of photodamage at months 4 through 24 compared with placebo ($p < 0.05$), and significant improvement in the investigator's global assessment of clinical response at all assessments (months 6, 12, 18, and 24) relative to placebo ($p < 0.02$).

The type and frequency of adverse events and cutaneous treatment effects in this study were consistent with the established safety profile of topical tretinoin products. Most signs and symptoms of cutaneous irritation were judged by the investigator to be mild. The majority (≥85%) of subjects in the study exhibited no change in histologic safety variables (keratinocytic and melanocytic dysplasia, dermal elastosis, and stratum corneum) during the 2-year study. Further, no significant differences between active and placebo treatment in the change from baseline to month 24 were detected for any of these histologic variables ($p > 0.05$). The histologic results confirm and expand data from previous studies.[11,13,14] Early reports included an extension study of subjects who completed 24 weeks of therapy with tretinoin and continued on the same regimen for an additional 24 weeks,[13] as well as a follow-up of 27 subjects from the original study who were treated

with tretinoin for up to 4 years.[14] There was no clinically meaningful evidence of keratinocytic or melanocytic atypia in these studies. Further, there was a decrease in the mean number of melanocytes in the 4-year biopsies compared with baseline biopsies, suggesting that long-term treatment with topical tretinoin does not induce melanocytic proliferation. Although these studies provided useful histologic information about the long-term safety of tretinoin, they did not include a control group, and the number of subjects evaluated was small. Data from the large-scale, placebo-controlled, multicenter trial described here provide definitive evidence that there are no untoward histologic effects on keratinocytes or melanocytes in patients treated with topical tretinoin for up to 2 years.

Previous studies demonstrated that 3–6 months of topical tretinoin therapy produces dose-dependent improvement in the epidermis of photodamaged skin, including increased epidermal thickness, increased granular layer thickness, decreased melanin content, and stratum corneum compaction.[11] However, after 12 months of therapy, the epidermal hyperplasia, increased granular layer thickness, and appearance of compact orthokeratosis seen at 6 months returned to baseline levels, despite the persistence of clinical benefits in skin roughness and fine wrinkling.[13] Nonetheless, there was a continued reduction in epidermal melanin content through 48 weeks, which was consistent with clinical improvement in dyspigmentation. Similar results suggesting a reversal of some histologic effects were reported in patients treated with topical tretinoin for up to 22 months.[8] The relationship between histologic changes and clinical improvement remains unclear. However, the 4-year follow-up of 27 patients showed a decrease in dermal elastosis and perivascular inflammation together with an increase in epidermal mucin,[14] suggesting that these changes may be of greater clinical significance than the transient changes observed during early treatment with topical tretinoin.

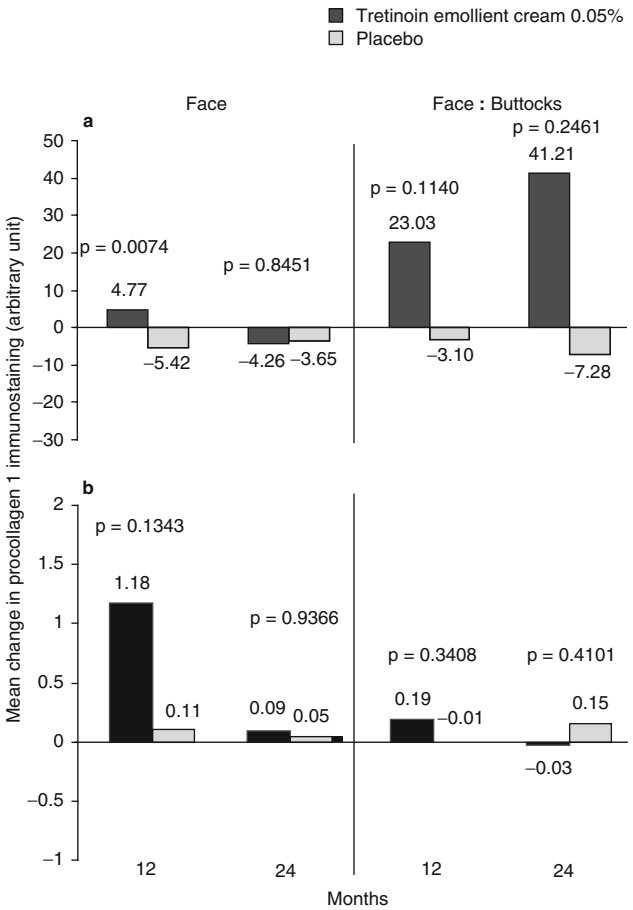


Fig. 5. Mean changes from baseline in facial procollagen levels and face-to-buttocks procollagen ratios following biopsies at months 12 and 24. (**a**) procollagen 1 C-terminal (PIC) levels. (**b**) procollagen 1 N-terminal (SP1) levels.

Recent publications have shed light on the pathophysiology of photodamage. Chen et al.[15] demonstrated that the ability of tretinoin to improve wrinkling in mice with photodamage correlated with increased collagen synthesis but not with epidermal hyperproliferation. Griffiths et al.[16] showed that collagen I formation was significantly decreased in the papillary dermis of photodamaged human skin relative to sun-protected skin. Consistent with the results observed in mice, treatment of human photodamaged skin with tretinoin produced an 80% increase in collagen I formation, whereas treatment with vehicle produced a 14% decrease in collagen formation.[16] Fisher et al.[17] demonstrated that exposure of human skin to ultraviolet radiation induced the synthesis of matrix metalloproteinases in the epidermis and dermis that degrade skin collagen. In the same study, treatment of human skin with tretinoin before exposure to ultraviolet irradiation inhibited the induction of matrix metalloproteinases. Several studies have shown that type I and type III procollagen production is inhibited in human photodamaged skin relative to sun-protected skin, suggesting that new synthesis of collagen to replace the damaged collagen is reduced in photodamaged skin.[18,19] In our study, facial biopsies of subjects treated with tretinoin tended to show an increase in immunohistologic indicators of procollagen synthesis. A significant increase in PIC ($p = 0.0074$) and a numerical increase in SP1 were observed at 12 months. Synthesized procollagen I is expected to be processed and incorporated into mature collagen matrix. Indeed, doubling of the thickness of the papillary dermal collagen band has recently been demonstrated in photoaged human skin following extended tretinoin treatment.[20] As more mature, cross-linked collagen is laid down with tretinoin therapy, it is plausible that further procollagen synthesis becomes downregulated. This may explain the lack of sustained procollagen I synthesis observed in our study at the 2-year time point.

Table III. Summary of adverse events occurring in ≥5% of subjects in either treatment group

Adverse events	Tretinoin, n (%) [n = 101]	Placebo, n (%) [n = 103]
No. of subjects with any adverse event	84 (83)	84 (82)
Event		
skin irritation	25 (25)	10 (10)
upper respiratory tract infection	14 (14)	20 (19)
dermatitis	12 (12)	12 (12)
influenza	11 (11)	5 (5)
sinusitis	11 (11)	4 (4)
bronchitis	7 (7)	5 (5)
solar keratosis	6 (6)	6 (6)
dry skin	9 (9)	0 (0)
herpes simplex	6 (6)	2 (2)
hypercholesterolemia	2 (2)	6 (6)

Conclusion

The results of this study demonstrate the long-term efficacy and safety of tretinoin emollient cream 0.05% in the treatment of subjects with moderate to severe facial photodamage. There was no evidence that treatment with tretinoin for up to 2 years caused an increase in keratinocytic or melanocytic atypia, dermal elastosis, or untoward effects on the epidermis relative to placebo.

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References

1. Chen VL, Fleischmajer R, Schwartz E, et al. Immunochemistry of elastotic material in sun-damaged skin. J Invest Dermatol 1986; 87: 334-7
2. Mera SL, Lovell CR, Jones RR, et al. Elastic fibres in normal and sun-damaged skin: an immunohistochemical study. Br J Dermatol 1987; 117: 21-7
3. Schwartz E, Cruickshank FA, Christensen CC, et al. Collagen alterations in chronically sun-damaged human skin. Photochem Photobiol 1993; 8: 841-4
4. Bernstein EF, Chen YQ, Kopp JB, et al. Long-term sun exposure alters the collagen of the papillary dermis: comparison of sun-protected and photoaged skin by northern analysis, immunohistochemical staining, and confocal laser scanning microscopy. J Am Acad Dermatol 1996; 34: 209-18
5. Weiss JS, Ellis CN, Headington JT, et al. Topical tretinoin improves photoaged skin: a double-blind vehicle-controlled study. JAMA 1988; 259: 527-32
6. Leyden JJ, Grove GL, Grove MJ, et al. Treatment of photodamaged facial skin with topical tretinoin. J Am Acad Dermatol 1989; 21: 638-44
7. Lever L, Kumar P, Marks R. Topical retinoic acid for treatment of solar damage. Br J Dermatol 1990; 122: 91-8
8. Ellis CN, Weiss JS, Hamilton TA, et al. Sustained improvement with prolonged topical tretinoin (retinoic acid) for photoaged skin. J Am Acad Dermatol 1990; 23: 629-37
9. Weinstein GD, Nigra TP, Pochi PE, et al. Topical tretinoin for treatment of photodamaged skin: a multicenter study. Arch Dermatol 1991; 127: 659-65
10. Olsen EA, Katz HI, Levine N, et al. Tretinoin emollient cream: a new therapy for photodamaged skin. J Am Acad Dermatol 1992; 26: 215-24
11. Bhawan J, Gonzalez-Serva A, Nehal K, et al. Effects of tretinoin on photodamaged skin: a histologic study. Arch Dermatol 1991; 127: 666-72
12. Thorne EG. Long-term clinical experience with a topical retinoid. Br J Dermatol 1992; 127: 31-6
13. Bhawan J, Palko M, Lee J, et al. Reversible histologic effects of tretinoin on photodamaged skin. J Geriatr Dermatol 1995; 3: 62-7
14. Bhawan J, Olsen E, Lufrano L, et al. Histologic evaluation of the long term effects of tretinoin on photodamaged skin. J Dermatol Sci 1996; 11: 177-82
15. Chen S, Kiss I, Tramposch KM. Effects of all-trans retinoic acid on UVB-irradiated and non-irradiated hairless mouse skin. J Invest Dermatol 1992; 98: 248-54
16. Griffiths CEM, Russman AN, Mjmudar G, et al. Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). N Engl J Med 1993; 329: 530-5
17. Fisher GJ, Wang ZQ, Datta SC, et al. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 1997; 337: 1419-28
18. Talwar HS, Griffiths CE, Fisher GJ, et al. Reduced type I and type III procollagens in photodamaged adult human skin. J Invest Dermatol 1995; 105: 285-90
19. Varani J, Spearman D, Perone P, et al. Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. Am J Pathol 2001; 158: 931-42
20. Cho S, Lowe L, Hamilton TA, et al. Long term treatment of photoaged human skin with topical retinoic acid improves epidermal cell atypia and thickens the collagen band in papillary dermis. J Am Acad Dermatol. In press

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