Histologic Improvement in Photodamage After 12 Months of Treatment With Tretinoin Emollient Cream (0.02%) 4806

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Introduction

- Exposure to sun or ultraviolet (UV) radiation is associated with changes in the skin.1–4
  - The typical histologic features of photodamaged skin include dermal elastosis, atypical epidermal cells, skin thinning and atrophy, glycosaminoglycan deposition, loss of cellular polarity, and decreased collagen.1–4
  - Topical tretinoin (0.02% to 0.1%) applied for 4-12 months has been shown to be effective for improving the clinical appearance of photodamaged skin.1–9
  - Histologic analyses have confirmed these findings by reducing demonstrated collagen loss, augmented collagen formation, and mitigation of solar elastosis with topical tretinoin (0.05% and 0.1%) for 6-12 months in patients with mild to moderate photodamage.10,11

Objective

- The aim of this pilot study was to evaluate histologic changes after 12 months of applying topical tretinoin emollient cream 0.02% to treat moderate to severe facial photodamage.

Methods

- This was a histologic subsanalysis that included 3 of 19 subjects who consented to skin biopsies, participating in a single-center, open-label, single-group observational study that evaluated the safety and efficacy of tretinoin emollient cream 0.02% for treating facial photodamage.12
- Subjects were 18 years of age or older with moderate to severe photodamage as classified by investigator ratings on the Glogau Photodamage Classification Scale.17
- Exclusion criteria were:
  - Female subjects who were pregnant, lactating, or of childbearing potential and not using a reliable method of birth control
  - Allergy or sensitivity to any component of the tretinoin medication
  - Recent alcohol or drug abuse
  - Medical contraindications
  - History of poor cooperation, noncompliance, or unrelievability
  - Participation in another investigational drug study within 30 days of baseline
  - Noncompliance with washout periods for prohibited medications/treatments (topical retinoids, topical steroids, prescription topical medications, nonprescription topical skin care products containing glycolic acid or salicylic acid, laser treatments, chemical peels, microdermabrasion, and cryotherapy)

Treatment

- Subjects were instructed to apply tretinoin emollient cream 0.02% for 12 months.
- A 2-week period of abstaining from tretinoin was applied each morning and evening 20 minutes after gentle cleansing of the facial treatment area with mild soap and water and application of a moisturizing sunscreen (SPF 15).
- Subjects were advised not to wash their faces again or to apply any other skin care products or cosmetics for at least 1 hour after the application.
- Facial exposure to sunlight and sunlamps was prohibited for the duration of the study.

Histologic Procedures

- Facial photographs and 2-mm punch biopsies were obtained at baseline and at the 12-month (final) study visit.
- All biopsy samples were taken from the lateral canthus area to ensure that the samples were collected from the midface.
  - Biopsy samples were fixed in 10% neutral buffered formalin, 4-µm sections were cut, and each section was stained as follows:
    - Hematoxylin and eosin (H & E), to differentiate the nucleus, melanin, and cytoplasm of the cell
    - Elastica-van Gieson (EvG), to specify elastic fibers and collagen
    - Masson's trichrome, to specify the type of collagen
    - Alcian blue, to differentiate glycosaminoglycans
- Each sample was evaluated for epidermal and papillary dermal thickness, presence of rete-ridge/papillary pegs at the epidermal/dermal junction, melanin content, and appearance of elastic and collagen fibers in the entire dermis.
- A single histologist who was not blinded to the time point (ie, baseline vs 12-month samples) evaluated each specimen with conventional light microscopy with and without polarized light at objective magnifications of x 4, x 10, and x 40.

Results

Summary of Findings

Baseline
- There was evidence of solar elastosis in all 3 subjects at baseline, which was extensive for Subjects 1 and 2 (white) and moderate for Subject 3 (African American).
- All 3 subjects had thin, horizontal collagen fibers in the superficial and middermis regions but retained normal interweaving or basket-wave pattern in the deep dermis.

12 Months
- All 3 subjects showed smoothing of the epidermis and a slightly thinner keratin layer.
- The thickened, elastic fibers observed at baseline had aggregated at the epidermal/dermal junction.
- Elastic and collagen fibers were also thinner and more normally oriented in the mid to deep derm.

Detailed Findings in Individual Patients

Subject 1
- Baseline: H & E staining revealed lightly keratinized evidence of rete-ridges, invaginated by pegs of fine collagen and small vessels from the adjacent papillary dermis. The normal basket-wave pattern of type I collagen in the upper reticular dermis was disrupted and could be associated with solar elastosis (Figure 1A). At 12 months: The epidermal layer was mainly smooth with no evidence of the unevenness seen at baseline. Also, there were no epithelial rete-ridges and papillary pegs, with little or no distinction between the dermal structure of the papillary and reticular layers (Figure 1B).
- All 3 subjects had thin, horizontal collagen fibers in the superficial and middermis regions but retained normal interweaving or basket-wave pattern in the deep dermis.

Subject 2
- Baseline: EVG-stained 4-µm section of photodamaged skin at baseline (A) and following 12 months of treatment with tretinoin emollient cream 0.02% (B) for Subject 1.
- Baseline: EVG staining confirmed the presence of collagen fibers extending from the papillary dermis to the deep-dermal junction (Figure 2A). At 12 months: EVG staining also showed a smoother epidermal layer, no evidence of pegs and rete-ridges, and the elastic fibers aggregated into clumps or coils in the papillary and upper reticular dermal layer (Figure 2B). In addition, there was a more homogeneous area below the mass of fibers due to apparent fibers being fixed in the fine reticular fibers that supported the blood vessels and skin appendages.

Subject 3
- Baseline: EVG-stained 4-µm section of photodamaged skin at baseline (A) and following 12 months of treatment with tretinoin emollient cream 0.02% (B) for Subject 1.
- Baseline: EVG staining confirmed the presence of collagen fibers extending from the papillary dermis to the deep-dermal junction (Figure 2A). At 12 months: There was a decrease in the level of elastin (relative to baseline images; Figure 4B). Elastic fibers were thin, straight, and normal in appearance and ran parallel to the epidermal surface.
- Unlike Subjects 1 and 2, there was no evidence of the amorphous gel possibly associated with mucopolysaccharides deposits.

Discussion

- This histologic subsanalysis showed that topical treatment with tretinoin emollient cream 0.02% for 12 months can affect dermal markers of photoaging.
- Histologic improvements observed in photoaged skin at 12 months included epidermal smoothing, thinning of the keratin layer, strengthening of elastic and collagen fibers in the mid- to deep-dermal layer, and a loss of rete-ridge/papillary peg complexes.
- The dense aggregates of thickened elastic fibers formed at the epidermal/dermal junction may have provided support for the regeneration of epithelial layer by the end of treatment.
- Additional histologic evaluation in a larger sample and for a longer treatment period is warranted.

References

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Introduction

• Exposure to sun or sources of ultraviolet (UV) radiation are associated with changes in the skin.\(^1\)\(^-\)\(^6\)
  - The typical histologic features of photoaged skin include dermal elastosis, atypical epidermal cells, skin thinning and atrophy, glycosaminoglycan deposition, loss of cellular polarity, and decreased collagen.\(^1\)\(^-\)\(^6\)

• Topical tretinoin (0.02% to 0.1%) applied for 4-12 months has been shown to be effective for improving the clinical appearance of photodamaged skin.\(^2\)\(^-\)\(^14\)

• Histologic analyses have confirmed these findings by demonstrating reduced collagen loss, augmented collagen formation, and mitigation of solar elastosis with topical tretinoin (0.05% and 0.1%) for 6-12 months in patients with mild to moderate photodamage.\(^11\)\(^,\)\(^15\)\(^,\)\(^16\)

Objective

• The aim of this pilot study was to evaluate histologic changes after 12 months of applying topical tretinoin emollient cream 0.02% to treat moderate to severe facial photodamage.
Methods

Patients
• This was a histologic subanalysis that included 3 of 19 subjects who consented to skin biopsies, participating in a single-center, open-label, single-group observational study that evaluated the safety and efficacy of tretinoin emollient cream 0.02% for treating facial photodamage.17

- Subjects were 18 years of age or older with moderate to severe photodamage as classified by investigator ratings on the Glogau Photodamage Classification Scale.17

- Exclusion criteria were:
  • Female subjects who were pregnant, lactating, or of childbearing potential and not using a reliable method of birth control
  • Allergy or sensitivity to any component of the tretinoin medication
  • Recent alcohol or drug abuse
  • Medical contraindications
  • History of poor cooperation, noncompliance, or unreliability
  • Participation in another investigational drug study within 30 days of baseline visit
  • Noncompliance with washout periods for prohibited medications/treatments (topical retinoids, topical steroids, prescription topical medications, nonprescription topical skin care products containing glycolic acid or salicylic acid, laser treatments, chemical peels, microdermabrasion, and cryotherapy)

Treatment
• Subjects were instructed to apply tretinoin emollient cream 0.02% for 12 months.

- A pea-sized amount of tretinoin was applied each morning and evening 20 minutes after gentle cleansing of the facial treatment area with mild soap and water and application of a moisturizing sunscreen (SPF ≥15).

- Subjects were advised not to wash their faces again or to apply any other skin care products or cosmetics for at least 1 hour after this application.

• Facial exposure to sunlight and sunlamps was prohibited for the duration of the study.
Methods
Histological Procedures
• Facial photographs and 2-mm punch biopsies were obtained at baseline and at the 12-month (final) study visit.
• All biopsy samples were taken from the lateral canthus area to ensure that the samples were collected from similar regions, with the baseline and 12-month biopsy sites adjacent to each other.
• Biopsy specimens were coded, and representative gross cuts of each specimen were fixed in 10% neutral buffered formalin, 4-µm sections were cut, and each section was stained as follows:
  - Hematoxylin and eosin (H & E), to differentiate the nucleus, melanin, and cytoplasm of the cell
  - Verhoeff’s Van Gieson (EVG), to specify elastic fibers and collagen
• Each sample was evaluated for epidermal and papillary dermal thickness, presence of rete-ridge/papillary pegs at the epidermal/dermal junction, melanin content, and appearance of elastic and collagen fibers in the entire dermis.
• A single histologist who was not blinded to the time point (ie, baseline vs 12-month samples) evaluated each specimen with conventional light microscopy with and without polarized light at objective magnifications of x 4, x 20, and x 40.

Results
Summary of Findings
Baseline
• There was evidence of solar elastosis in all 3 subjects at baseline, which was extensive for Subjects 1 and 2 (white) and moderate for Subject 3 (African American).
• All 3 subjects had thin, horizontal collagen fibers in the superficial and middermis regions but retained normal interwoven or basket-weave pattern in the deep dermis.

12 Months
• All 3 subjects showed smoothing of the epidermis and a slightly thinner keratin layer.
• The thickened, elastic fibers observed at baseline had aggregated at the epidermal/dermal junction.
• Elastic and collagen fibers were also thinner and more normally oriented in the mid to deep dermis, and there was an absence of rete-ridge pegs.
Results
Detailed Findings in Individual Patients
Subject 1

• **Baseline:** H & E staining revealed lightly keratinized evidence of rete-ridges, invaginated by pegs of fine collagen and small vessels from the adjacent papillary dermis. The normal basket-weave pattern of type I collagen in the upper reticular dermis was disrupted and could be associated with solar elastosis (Figure 1A).

• **12 months:** The epidermal layer was mainly smooth with no evidence of the unevenness seen at baseline. Also, there were no epithelial rete-ridges and papillary pegs, with little or no distinction between the dermal structure of the papillary and reticular layers (Figure 1B). Fine collagen fibers ran parallel to the epidermal layer, suggesting new collagen formation.

Figure 1. H & E–stained 4-µm section of photodamaged skin at baseline (A) and following 12 months of treatment with tretinoin emollient cream 0.02% (B) for Subject 1.
Results

• **Baseline:** EVG staining confirmed the extensive solar elastosis that was suggested by the H & E staining. The thickened and irregular elastic fibers tended to be vertically oriented (Figure 2A).

• **12 months:** EVG staining also showed a smoother epidermal layer, no evidence of pegs and rete-ridges, and the elastic fibers aggregated into clumps or coils in the papillary and upper reticular dermal layer (Figure 2B).

- In addition, there was a moderate-sized area below this mass of fibers that appeared to be filled with amorphous gel, possibly attributed to condensation or increased mucopolysaccharide deposits.

- Evidence of collagen was found in the fine reticular fibers that supported the blood vessels and skin appendages.

Subject 2

• EVG and H & E specimens from Subject 2 were very similar to those for Subject 1 (and will not be illustrated further here).
Results

Subject 3

- **Baseline:** H & E specimens showed a thin and lightly keratinized epidermis, with evidence of solar elastosis extending from the papillary dermis to the mid dermis (Figure 3A).
  - Collagen bundles in the papillary and mid dermis were relatively straight, and fibers were arranged in a basket-weave pattern in the deep dermis.
  - There was a single rete-ridge/papillary peg showing normal, fine, and vertically oriented collagen fibers within the peg, whereas the remaining collagen fibers in the papillary dermis ran parallel to the epidermis.

- **12 months:** There was a complete absence of the rete-ridge/peg complex observed at baseline and a decrease in solar elastosis (Figure 3B). Collagen fibers in the papillary and mid dermis and elastic fibers predominantly ran parallel to the epidermal surface. A more normal interwoven pattern of collagen fibers was found in the deep dermis.

Figure 3. H & E–stained 4-μm section of photodamaged skin at baseline (A) and following 12 months of treatment with tretinoin emollient cream 0.02% (B) for Subject 3.
• **Baseline:** EVG staining confirmed solar elastosis extending from the papillary dermis to the deep-dermal subcutaneous junction (Figure 4A). Elastic fibers were thickened and wavy and tended to be distributed vertically rather than horizontally. There were few collagen fibers.

• **12 months:** There was a decrease in the level of elastosis (relative to baseline images; Figure 4B). Elastic fibers were thin, straight, and normal in appearance and ran parallel to the epidermal surface.

- Unlike Subjects 1 and 2, there was no evidence of the amorphous gel possibly associated with mucopolysaccharide deposits.

![Figure 4. EVG-stained 4-µm section of photodamaged skin at baseline (A) and following 12 months of treatment with tretinoin emollient cream 0.02% (B) for Subject 3.](image)

**Conclusions**

• This histologic subanalysis showed that topical treatment with tretinoin emollient cream 0.02% for 12 months can affect dermal markers of photoaging.

• Histologic improvements observed in photoaged skin at 12 months included epidermal smoothing, thinning of the keratin layer, straightening of elastin and collagen fibers in the mid- to deep-dermal layer, and a loss of rete-ridge/papillary peg complexes.

- The dense aggregates of thickened elastic fibers formed at the epidermal/dermal junction may have provided support for the regeneration of epithelial layer by the end of treatment.

• Additional histologic evaluation in a larger sample and for a longer treatment period is warranted.
References


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