Skin Rejuvenation Regimens

A Profilometry and Histopathologic Study

John D. Rachel, MD; Jasmin J. Jamora, MD

Objective: To quantitatively examine the effects of skin rejuvenation regimens in treating photodamaged skin.

Methods: Fourteen patients with photodamaged skin were considered for analysis. Skin rejuvenation regimens were as follows: (1) 10 weeks of treatment with topical 0.05% tretinoin emollient cream, (2) 10 weeks of treatment with 0.05% tretinoin emollient cream and topical ascorbic acid lotion, (3) 6 superficial trichloroacetic acid peels, and (4) a combination of the topical treatments and superficial peels. Comparisons of the treatments were analyzed using profilometry and histologic findings.

Results: Profilometry analysis provided quantification of the changes from each treatment group and among the treatment programs. Each group showed improvements from baseline. Trichloroacetic acid peels combined with application of the topical products improved skin topography to a greater extent than the less aggressive regimens. Histologic changes correlated well with the skin replica findings.

Conclusions: A 10-week skin rejuvenation regimen objectively improved photodamaged facial skin. Significant changes are noted when combining topical treatments with superficial peels. Hence, use of a combination of trichloroacetic acid peels, 0.05% tretinoin emollient cream, and ascorbic acid lotions is well tolerated and superior to either component alone as part of a comprehensive skin care and sun protection program.

Arch Facial Plast Surg. 2003;5:145-149

Photodamaged skin is characterized by fine and coarse wrinkling, rough texture, sallow color, and uneven pigmentation. These characteristics are associated with decreased microcirculation, elastosis, epidermal atrophy, cellular atypia, and preneoplastic dysplasia. Clinical skin changes presumably result from fibroblast malfunction secondary to DNA and other cellular and protein damage. The consequence is abnormal collagen, elastin, and ground substance breakdown and resynthesis and, thus, abnormal dermal structure repair and remodeling. In addition to actinic keratosis and skin cancer, these changes predispose to decreased immune function and vitamin D synthesis and poor wound healing. Long-term exposure to natural and synthetic stimuli, including UV light, ozone, and tobacco smoke, can lead to the development of photoaging.

The main pharmaceutical approach to the prevention of photoaging lies in the assiduous use of sunscreens. Many products are available to reverse the damaging effects of the sun and help rejuvenate the skin, including vitamin A, ascorbic acid derivatives, and chemical peels. A frequently prescribed treatment regimen includes combinations of these products.

Results of some studies have shown that the topical application of tretinoin, a vitamin A derivative, is effective in reversing some changes in photoaging. These changes include increased number and activity of fibroblasts, reduced melanocyte activity, and rapid formation of a zone of subepidermal connective tissue with new collagen and anchoring fibrils. This reversal reduces in fine wrinkling, roughness, and mottled hyperpigmentation. Studies using skin replica analysis and histologic evaluation have confirmed the tretinoin-associated improvements.

Oxidative damage to the skin is produced by reactive oxygen species such as free radicals. Ascorbic acid is an antioxidant that neutralizes oxygen and free radicals and has a role in collagen stimulation. Cutaneous stores of ascorbic acid are depleted by UV light exposure. Oral ingestion of the vitamin has been ineffective in achieving adequate levels in the skin. Topical application of ascorbic acid can increase the cutaneous levels by more than 20 times the amount found in normal skin.
stable form of L-ascorbate has allowed pharmacologic levels of ascorbic acid penetration targeted directly into the skin by topical application to affect the free radicals and stimulate collagen. Skin replica analysis and clinical assessments of ascorbic acid–treated skin have shown significant improvements in skin surface texture and tone.

A third modality used in the armamentarium for skin rejuvenation programs is chemical peeling agents. The peel is intended to produce a controlled partial-thickness skin injury, destroying varying amounts of epidermis and upper portions of the dermis. Trichloroacetic acid peels cause wounding through protein precipitation. A wound-healing response after injury includes an increase in glycosaminoglycan content and qualitative changes in dermal collagen. The overall clinical appearance of the skin is more homogenous, with fewer rhytids and less pigmentation dyschromia. Chemical peeling agents can be classified by the depth of penetration: superficial, medium, and deep. This classification is based on studies showing the depth of wound produced when the agents are applied in controlled experimental situations. Many agents are available for superficial chemical peels, including a 10% to 25% solution of trichloroacetic acid. These agents are generally well tolerated, and the potential for systemic toxic effects is minimal.

The clinical changes resulting from application of these products have been studied individually in the past. Few studies comparing skin rejuvenation programs have been reported. This study aims to quantify the changes in skin surface topography in patients undergoing routine skin care treatment with combined topical 0.05% tretinoin emollient cream, topical ascorbic acid, and superficial trichloroacetic acid peels.

**METHODS**

Fourteen patients were enrolled in this 10-week prospective study carried out in a private facial plastic surgery practice and ambulatory surgery center (Beeson Aesthetic Surgery Institute, Indianapolis, Ind) fully accredited by the Accreditation Association for Ambulatory Health Care Inc. Twelve of the patients were randomly assigned to 4 study groups. Group A received daily topical application of 0.05% tretinoin emollient cream (Renova; Ortho Dermatological, Skillman, NJ). Group B applied 0.05% tretinoin emollient cream each evening and topical ascorbic acid lotion (Cellex-C High-potency serum; Cellex-C International, Toronto, Ontario) each morning. Group C received 6 weekly superficial trichloroacetic acid peels. The first 3 peels were performed using a 10% solution and the last 3 a 20% solution. Group D received a combination of the group B and group C regimens. The peels started 2 weeks after initiation of 0.05% tretinoin and ascorbic acid application. The creams were applied daily to the entire facial area for 10 weeks except on the days on which the peels were received. Two additional patients who agreed to undergo postauricular biopsies at completion of the study were included in group D.

Patients were allowed to use a moisturizer and a mild soap ad libitum and were cautioned against sun exposure during the study. Patients were given verbal and written instructions for application of the topical products in the postauricular region. Product application followed the regimens of the 4 treatment groups. Postauricular site 1 received 0.05% tretinoin emollient cream alone, site 2 received 0.05% tretinoin emollient cream and ascorbic acid, site 3 received trichloroacetic acid alone, and site 4 received the trichloroacetic acid peels and 0.05% tretinoin emollient cream and ascorbic acid. At completion of the study, a 4-mm round, stainless steel, trephine punch biopsy was used to obtain a sample at each site. The biopsy tissue specimen was fixed and examined by light microscopy. The dermatopathologist (J.J.J.) was masked to the origin of each biopsy specimen. The sections were prepared in the usual manner with hematoxylin-eosin to observe cellular response and with van Gieson stain to observe elastic tissue response.

**HISTOLOGIC ANALYSIS**

Histologic analyses of postauricular punch biopsy samples were performed to further analyze the skin care regimens. Templates made of radiographic paper were fashioned to facilitate precise application of the topical products and to serve as a reference for biopsy site identification. Patients were given verbal and written instructions for application of the topical products in the postauricular region. Product application followed the regimens of the 4 treatment groups. Postauricular site 1 received 0.05% tretinoin emollient cream alone, site 2 received 0.05% tretinoin emollient cream and ascorbic acid, site 3 received trichloroacetic acid alone, and site 4 received the trichloroacetic acid peels and 0.05% tretinoin emollient cream and ascorbic acid. At completion of the study, a 4-mm round, stainless steel, trephine punch biopsy was used to obtain a sample at each site. The biopsy tissue specimen was fixed and examined by light microscopy. The dermatopathologist (J.J.J.) was masked to the origin of each biopsy specimen. The sections were prepared in the usual manner with hematoxylin-eosin to observe cellular response and with van Gieson stain to observe elastic tissue response.

**ANALYTICAL AND STATISTICAL METHODS**

Patient data were analyzed by calculating the difference between the baseline and week 10 measurements for each variable (RaNS, RaEW, RzNS, RzEW, shadow NS, and shadow EW). A negative result indicates improvement. A t test was used to analyze each variable and to analyze changes between groups and was considered statistically significant at P<.05. A corre-
RESULTS

Fourteen patients were included in the study, and each completed the 10-week course of topical product application. No patients were excluded from analysis. All patients were women aged 36 to 61 years (mean ± SD age, 47.5 ± 5.7 years). Four patients (29%) regularly used sun precautions, and 6 (42%) reported a history of tobacco use. Fitzpatrick skin type was II in 3 patients (21%), III in 8 (57%), and IV in 3 (21%). Glogau classification was II in 2 patients (14%), III in 11 (78%), and IV in 1 (7%). The treatment groups were comparable at baseline with respect to skin replica measurements and clinical characteristics, including overall severity of photodamage. Adverse effects were mild, resolved within the first week of therapy, and included stinging, erythema, and dryness, which were easily treated with moisturization. In no case was the study regimen altered.

Profilometric data were obtained from skin replica analysis at baseline and at the end of the study. Each patient provided 2 sets of data. Table 1 summarizes the correlation coefficients in the population. Analysis showed the treatment groups to be comparable at baseline and indicates strong reproducibility between sides.

Table 1. Correlation Data for Profilometric Variables

<table>
<thead>
<tr>
<th>Correlation Coefficient</th>
<th>Patients, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between right and left sides</td>
<td></td>
</tr>
<tr>
<td>$R &gt; 0.90$</td>
<td>9 (64)</td>
</tr>
<tr>
<td>$0.75 &lt; R &lt; 0.90$</td>
<td>2 (14)</td>
</tr>
<tr>
<td>$R &lt; 0.75$</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Between groups at baseline</td>
<td></td>
</tr>
<tr>
<td>$R &gt; 0.98$</td>
<td>14 (100)</td>
</tr>
</tbody>
</table>

Table 2 summarizes the mean change from baseline for each variable in each group. Improvement in skin topography, measured as a mean decrease from baseline to week 10 in the Ra and Rz values, occurred in each of the 4 treatment groups. Statistically significant changes from baseline for all 4 groups were found for the RaNS, RzNS, and RzEW variables. The RaEW value was significantly improved from baseline in groups B and D. Shadow NS showed significant changes in groups A and D. There were no significant changes in the shadow EW variables.

Figure 1 illustrates the differences among the 4 groups for each of the 6 variables. Treatment in group A patients resulted in small (0 to −2) decreases in 3 variables, mild (−2 to −4) decreases in 2, and moderate (−4 to −6) decreases in 1. In group B, results indicate relatively small to moderate decreases across the variables, except for RzEW, which shows a large (>−6) mean change. Groups C and D experienced the greatest changes, with half of the variables demonstrating moderate to large increases. The effects of trichloroacetic acid peels alone and in combination with application of the 2 topical products showed the greatest reductions from baseline in the NS and EW axis of the Rz variable.

The differences among patients in groups B, C, and D compared with group A showed statistically significant improvements in the Rz variables. The EW axis was significant for groups B (P = .03), C (P = .05), and D (P < .005), and the NS axis was significant for groups C (P = .03) and D (P = .001). The differences were also large for RaEW when comparing groups A and D (P = .001). The Rz variables were significantly different as well when comparing groups C and D with group B. The NS axis showed large differences (groups C, P = .01; groups D, P = .009) for both groups and the EW axis showed significant changes for group D (P = .03). When comparing groups C and D, there were no significant differences in either the Ra or Rz variables. Although the mean changes for the shadow variables were small for all groups, some significant changes were noted. The NS variable was significantly different when comparing group B with group A and groups C and D with group B (P = .04 for all). The EW variable was significantly different only when comparing group D with group C (P = .05).

The histologic results, given as the average between the 2 patients except for the epidermal thickness measurements, show progressive changes from specimens 1 through 4 in both patients. The stratum corneum was mostly basket weave: +4 on a scale from 1 to 4 on specimen 1, which became less evident on specimens 2 and 3 and was nonexistent in specimen 4. Compact orthokeratosis was slightly present on specimen 1 (+1) but became progressively more evident on specimens 2 (+2), 3 (+3), and 4 (+4). These findings indicate definite changes from basket-woven orthokeratosis to compact orthokeratosis.

The granular cell layer was noted to be approximately 1 cell thick in specimen 1, and this was noted to progressively thicken, so that it was 2 cells thick by specimen 3 and 4 cells thick by specimen 4. The thickness of the epidermis was measured from the granular cell layer to the bottom of the interpapillary epidermis and aver-
aged across the front of the specimen. On patient 1, it was noted to be 0.06 mm on specimen 1, 0.09 mm on specimen 2, 0.10 mm on specimen 3, and 0.13 mm on specimen 4. Measurements on the second patient were 0.08 mm on specimen 1, 0.09 mm on specimens 2 and 3, and 0.10 mm on specimen 4. These results show an increased thickening of the epidermis on both specimens, progressing from sites 1 through 4.

These results show an increased thickening of the epidermis on both specimens, progressing from sites 1 through 4.

The elastic tissue, as demonstrated with the elastic tissue stain, showed progressive increases from specimens 1 to 4 (+2 in specimens 1 and 2, +3 in specimen 3, and +4 in specimen 4). There was also significant thickening in the papillary dermis (+2 at specimens 1, 2, and 3 and +4 at specimen 4). There was an increase in the number of lymphocytes noted around the capillaries from a factor of approximately 25%. The average perivascular lymphocytic infiltrate on specimen 1 was approximately 11 mononuclear cells, which increased to 12 for specimen 2, 13 for specimen 3, and 14 for specimen 4.

COMMENT

Combinations of therapeutic agents are often used in the treatment of photodamaged and chronically aged skin to allow synergy of mechanisms with tolerability. New understanding regarding the pathogenesis of aged skin has allowed the development of topical medications designed to optimize skin appearance when used in conjunction with proper skin care and sun protection routines. Little quantitative information exists in the literature about the concomitant use of these products.

The use of skin replica analysis in conjunction with histologic analysis provided an instrument to evaluate the changes in the photodamaged skin during the 10-week study. Studies6-8,15 have confirmed the value of optical profilometry as an objective technique that could reproducibly measure changes in skin topography with minimal variability or potential for bias. Grove et al6 performed skin replica analyses of photodamaged skin after therapy with tretinoin emollient cream, and improvements were found in a dose-related fashion. Use of 0.05% tretinoin emollient cream consistently gave greater reductions in Ra and Rz variables relative to vehicle. Data also showed greater changes in Ra and Rz with EW measurements than with NS measurements, supporting the clinical data showing a greater effect on superficial fine lines than on coarse wrinkles. The deeper crow’s feet have considerable impact on the NS measurements, whereas the contribution of fine lines on crow’s feet is relatively greater with the EW measurements.16

The present study supports 0.05% tretinoin emollient cream as an effective topical treatment for improving photodamaged skin. Significant changes from baseline were found in Ra and Rz variables, with the greatest changes in the EW axis. The addition of ascorbic acid and treatment with trichloroacetic acid peels alone resulted in similar findings, with significant changes in Ra and Rz variables, again with the greatest changes in the EW axis. This is consistent with findings from previous studies11,17,18 demonstrating improvement in fine wrinkling with the use of topical ascorbic acid and superficial trichloroacetic acid peels. Trakovich11 used optical profilometry and clinical analysis to determine the efficacy of topical ascorbic acid in treating mild to moderate photodamage. Clinical investigator assessment was consistent with findings from skin replica analysis showing greater improvement in fine wrinkling and a smoothening of the skin surface.

Group D profilometry results demonstrated significant changes from baseline (P<.005) across the NS and EW measurements of Ra and Rz variables. Data were similar for both measurements, indicating further improvement with the combination therapy. Examination of profilometric data between groups supports these findings.
RzEW was significantly different in groups B, C (P<.05), and D (P<.01) with use of 0.05% tretinoin alone. When trichloroacetic acid peels were performed (groups C and D), RzNS was also improved. These findings may initially indicate an additive effect of the peels to the skin care regimen. This effect is supported in comparison of 2 peel groups and the patients who applied tretinoin emollient cream and ascorbic acid, resulting in significant changes in the Rz variables. The additive effect remains questionable, however, when reviewing the group C and D comparisons. There were no significant differences in the Rz or Ra variables, although trends were noted in the RzNS (P<.06) and RzEW (P<.06) variables.

Histologically, several mechanisms have been proposed to account for the beneficial effects of skin rejuvenation products. A reorganization in dermal structural elements, collagen and elastin, and an increase in dermal volume have been found to restore skin structure and function. Researchers have previously reported that an increase in glycosaminoglycan content would obligate an influx of water into the dermis, increasing the volume and thus decreasing wrinkling.

The most notable early histologic findings in patients treated with tretinoin included increases in epidermal thickness, decreased melanocyte hypertrophy, and compaction of the basket-weave pattern of the stratum corneum. Histologic results from this study represent early changes and show progressive changes from groups A to D. Although these histologic findings are subtle, they were consistent with those of previous studies. In our series, the trichloroacetic acid peel groups experienced the histologic changes earlier than groups A or B. Slides from groups C and D showed increased perivascular lymphocytes around the capillaries, indicating a deeper and exaggerated wound- ing response relative to groups A and B. Treatment in the trichloroacetic acid groups compared with groups A and B would allow induction of an inflammatory reaction deeper in the tissue. This has been shown to create cumulative benefits through activation of the mediators of inflammation, inducing the production of collagen and ground substance (glycosaminoglycans) in the dermis.

These findings are encouraging, but limitations of this study include sample size and length of treatment. Length of treatment in studies of similar products varies greatly, providing data in the literature for the short- and long-term effects of these products on the skin. Another limitation is that biopsy samples were from skin that does not undergo similar chronic sun exposure as the face. This is of little consequence because the results are comparable to those of the other groups, and nontreated skin is not included as a biopsy site. Application of the topical products in the postauricular region was carefully reviewed with each patient, and templates were provided for consistency of placement and localization of biopsy sites. Product placement and timing of application was arranged to minimize agent crossover. However, one cannot completely control or measure patient compliance in these matters.

In conclusion, all test products modified some of the variables selected in the present study. The changes could all be interpreted as improvements for the skin conditions. Significant differences in efficacy were yielded between product combinations. In general, the use of 0.05% tretinoin emollient cream alone resulted in the weakest beneficial effect among the product groups. Trends were noted toward greater improvement with the addition of ascorbic acid lotion. Use of trichloroacetic acid peels resulted in the greatest changes in the study variables. The combination of trichloroacetic acid peels with the tretinoin emollient cream and ascorbic acid lotion achieved results as good as the best of its components, and application was tolerated well. Therefore, for the treatment of photoaging, the combination of these products is well tolerated and superior to either component as part of a comprehensive skin care and sun protection program.

Accepted for publication October 2, 2002.

This paper was selected for the American Academy of Facial Plastic and Reconstructive Surgery John Orlando Roe Award and was presented at the American Academy of Cosmetic Surgery 18th Annual Scientific Meeting, Ft Lauderdale, Fla, February 1, 2002.

Corresponding author and reprints: John D. Rachel, MD, 680 N Lake Shore Dr, Suite 1201, Chicago, IL 60611.

REFERENCES