Use of Topical Ascorbic Acid and Its Effects on Photodamaged Skin Topography

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Objective: To determine the efficacy of topical ascorbic acid application in treating mild to moderate photodamage of facial skin using an objective, computer-assisted image analysis of skin surface topography and subjective clinical, photographic, and patient self-appraisal questionnaires.

Design: A 3-month, randomized, double-blind, vehicle-controlled study.

Setting: Facial plastic surgery private practice.

Patients: Nineteen evaluable volunteer sample patients aged between 36 and 72 years with Fitzpatrick skin types I, II, and III who were in good physical and mental health with mild to moderately photodamaged facial skin were considered for analysis.

Intervention: Coded, unmarked medications were randomly assigned to the left and right sides of each subject's face, one containing the active agent, topical ascorbic acid (Cellex-C high-potency serum; Cellex-C International, Toronto, Ontario), the other, the vehicle serum (Cellex-C International). Three drops (0.5 mL) of each formulation were applied daily to the randomly assigned hemifaces over the 3-month study period. Treatment assignments were not disclosed to subjects, clinicians, or personnel involved in analyzing skin replicas.

Main Outcome Measures: Specific clinical parameters were evaluated and graded on a 0- to 9-point scale (0, none; 1-3, mild; 4-6, moderate; and 7-9, severe). Reference photographs were used to standardize grading criteria. Overall investigator scores were compared with baseline graded as excellent (much improved), good (improved), fair (slightly improved), no change, or worse. Patient self-appraisal questionnaires rated the degree of improvement (much improved, improved, slightly improved, no change, or worse) and reported adverse effects (burning, stinging, redness, peeling, dryness, discoloration, itching, and rash). Standard photographs were taken at baseline, including anteroposterior and left and right oblique views to facilitate subsequent clinical evaluations, and at the end of therapy for comparison. Optical profilometry analysis was performed on the skin surface replicas of the lateral canthal (crow's feet) region, comparing baseline to end-of-study specimens. Using this computer-based system, the resulting image was digitally analyzed, and numeric values were assigned to reflect surface features. The parameters obtained included \(R_z\), \(R_a\), and shadows. These values provided objective data that document pretreatment and posttreatment texture changes proportional to the degree of wrinkling, roughness, and other surface irregularities.

Results: Optical profilometry image analysis demonstrated a statistically significant 73.7% improvement in the \(R_z\) and shadows north-south facial axis values with active treatment greater than vehicle control, as well as a trend for improvement in the \(R_a\) north-south facial axis parameter, showing a 68.4% greater improvement of active treatment vs vehicle control. Clinical assessment demonstrated significant improvement with active treatment greater than control for fine wrinkling, tactile roughness, coarse rhytids, skin laxity/tone, sallowness/yellowing, and overall features. Patient questionnaire results demonstrated statistically significant improvement overall, active treatment 84.2% greater than control. Photographic assessment demonstrated significant improvement, active treatment 57.9% greater than control.

Conclusions: A 3-month daily regimen of topical ascorbic acid provided objective and subjective improvement in photodamaged facial skin. Skin replica optical profilometry is an objective method for quantification of the skin surface texture changes.


**From the Beeson Aesthetic Surgery Institute, Carmel, Ind. The financial disclosure statement of the author appears in the acknowledgment section at the end of the article.**
PATIENTS AND METHODS

PROCEDURES

Twenty-eight healthy volunteers were enrolled in this 3-month, randomized, double-blind, vehicle-controlled study. Coded, unmarked treatment preparations were randomly assigned to the left and right sides of each subject’s face: one containing the active agent, topical ascorbic acid (Cellex-C high-potency serum; Cellex-C International, Toronto, Ontario), and the other, the vehicle serum (Cellex-C International). The primary composition of the active and vehicle serums in this study included L-ascorbic acid, tyrosine, and zinc (active); and bioflavonoid, hyaluronic acid, and water base (vehicle), respectively. These formulations were matched for color, feel, and pH. Three drops (0.5 mL) of each formulation were applied daily over the randomly assigned hemifaces for 3 months. Treatment assignments were not disclosed to subjects, clinicians, or personnel involved in analyzing the skin replicas. Treatment bottles containing 3.7 mL of serum were given to the patients, and bottle counts were recorded to evaluate appropriate patient compliance. Sequential clinical analysis and patient questionnaires were performed at baseline, 2 weeks, 1 month, 2 months, and 3 months. In addition, optical profilometry measurements of silicone rubber casts were taken from identical sites at the lateral canthal (crow’s feet) areas. Standard photographs were taken at baseline and at the end of the study (3 months).

The specific clinical parameters evaluated were fine wrinkling, tactile roughness, visual dryness, coarse rhytids, telangiectasia, laxity/tone, pigmentation, keratoses, and sallowness/yellowing. Each of these parameters was graded on a 0- to 9-point scale (0, none; 1-3, mild; 4-6, moderate; and 7-9, severe). Reference photographs were used to standardize grading criteria. Overall investigator scores were compared with baseline and graded as excellent (much improved), good (improved), fair (slightly improved), no change, or worse. Patient self-appraisal questionnaires rated the degree of improvement (much improved, improved, slightly improved, no change, or worse) and reported adverse effects (burning, stinging, redness, peeling, dryness, discoloration, itching, and rash). Standard photographs were taken at baseline, including anteroposterior and left and right oblique views to facilitate subsequent clinical evaluations, and at the end of therapy for comparison. Optical profilometry analysis was performed on skin surface replicas of the crow’s feet region, comparing baseline with end-of-study specimens. Silicone skin surface replicas were taken from the periorbital crow’s feet region at identical sites bilaterally by the same technician.

The periorbital region was cleansed with alcohol before application of the adhesive rings and silicone impression material (Silfo; CuDerm Corporation, Dallas, Tex). Precise application of the adhesive replica locating rings was aided with caliper measurements to ensure consistent distances from reference points of the lateral orbital canthus and superior auricular tragus. This, as well as use of reference close-up Polaroid photographs with adhesive rings properly placed for each subject, facilitated relocating these sites for subsequent end-of-study comparison samples. The center hole of the adhesive rings was placed in the area of interest with the orientation of the ring tab facing outward, toward the ear. With the patient in a supine position, a thin layer of Silfo silicone impression material was applied over the bounded area of the ring and allowed to polymerize over a 3- to 4-minute period, after which the ring was lifted from the skin together with the replica. Each specimen was labeled with the date and patient’s identification number by posttranslational and transcriptional mechanisms. This is thought to occur as a result of ascorbate directly stimulating collagen synthesis, directly and specifically activating collagen gene regulation both by increasing the transcription rate and stabilizing procollagen messenger RNA, thus genetically signaling collagen synthesis. Another mechanism is initiation of lipid peroxidation, which leads to an increase in a by-product, malondialdehyde, which somehow stimulates collagen gene expression.

It has been proposed that ascorbate influences quantitative collagen synthesis in addition to stimulating qualitative changes to the collagen molecule. Ascorbic acid is necessary for the formation of prolyl hydroxylase, an enzyme essential for producing a stable collagen molecule. In addition, ascorbic acid is necessary to form lysyl hydroxylase, an enzyme necessary for cross-linking one collagen molecule to another, providing tissue strength.

Free radicals or reactive oxygen species created from endogenous (physiologic) sources such as mitochondrial electron transport chains and exogenous sources such as UV light exert an “oxidative stress” on the skin, which damages the DNA and/or protein. Ascorbate is the main water-soluble, nonenzymatic antioxidant. Ascorbic acid interacts with a variety of free radicals intracellularly and extracellularly and is one of the most efficient antioxidants in aqueous compartments.

Humans are one of the few species that require dietary supplementation of ascorbic acid for survival; our bodies do not produce this necessary vitamin. Without ingestion, ascorbic acid would be mostly depleted after 3 weeks. The minimum daily requirement for ascorbic acid is 200 mg. Unfortunately, UV light exposure depletes up to two thirds of cutaneous ascorbic acid stores, and oral ingestion is ineffective in achieving adequate cutaneous replenishment. Cutaneous levels not obtainable by ingestion, however, can be reached with topical application. A unique formulation of topical ascorbic acid consisting of L-ascorbate, tyrosine, and zinc has been shown to provide more than 20 times the amount of ascorbic acid found in normal skin. Ascorbic acid stereoisomers D-ascorbic acid and L-ascorbic acid exist, but the body can use only the L-ascorbic acid form.

Ascorbic acid is notoriously difficult to stabilize, and this has precluded its use as a general topical cosmetic ingredient. Duke University Medical Center, Durham NC, has recently developed and patented a stable topical formulation of L-ascorbic acid, which has allowed pharmacological levels of ascorbic acid to penetrate directly into the skin by topical application and to act as antioxidant and collagen stimulator. Zinc is necessary to help sup-
The resulting skin surface image was digitally analyzed, and numeric values were plotted to create profiles reflecting surface features at these specific locations. These plots yielded parameters (R,<sub>a</sub>, and shadows) proportional to the degree of wrinkling, roughness, and other surface irregularities. R<sub>a</sub> analysis divides the scan into 5 sections and looks at the minimum-maximum within each segment, calculating the average of these values; R<sub>a</sub> generates an average line through the center of the profile and determines the area above and below this line. Shadows represent the area of dark shadows seen with profilometric analysis. With these values, one can vary illumination from different orientations. North-south measurements are obtained with the illumination running perpendicular to the major line axis, whereas east-west measurements are made with parallel lighting.

To standardize topical skin product use, patients were supplied with a moisturizer (Eucerin; Beiersdorf Inc, Norwalk, Conn) and mild soap (Johnson’s Baby Bar; Johnson & Johnson Consumer Products Inc, Skillman, NJ) for routine use. All patients were instructed to continue using the same brand and quantity of makeup they used before the study. They did not apply cosmetics and/or makeup on days of clinical assessments. Patients were not to use topical and/or systemic tretinoin, α-hydroxy acids, kojic acid, hydroquinones, or steroids for at least 30 days before the study. Subjects did not have any history of previous laser resurfacing, chemical peels, dermabrasions, or other cutaneous facial surgery for at least 1 year before entering the study. A serious adverse reaction, patient noncompliance, and/or patient request resulted in discontinueation from the study. All subjects gave informed consent before the study. The project was approved by our institutional review board.

**ANALYTICAL AND STATISTICAL METHODS**

Skin surface impression data from week 0 (baseline), week 12, and changes from week 0 to week 12 were summarized for both active and control treatments. Changes were calculated by subtracting the week 0 result from the week 12 result; thus, negative results indicate improvement. The sign test was used to determine whether active treatment was more effective than the control on two of the 10 occasions that treatment was more effective than the active treatment. The clinical investigator assessment data were analyzed similarly (Figure). For the overall clinical assessment, patient questionnaire responses, photographic assessments, the percentages of subjects whose conditions worsened, had no change, slightly improved, improved, and were much improved were all summarized for the active treatment and vehicle control (Figure). As with the other data, the sign test was used to determine whether active treatment was more effective more often than control.

The sign test was performed using Proc Univariate in SAS, version 6.12.<sup>34</sup> Tests were 1-sided, and results were considered statistically significant if the P value was less than .05.
dermis and dermis. It is anticipated that this enhanced delivery technology, using a unique formulation of L-ascorbic acid, zinc sulfate, and L-tyrosine will provide an improved, longer-lasting effect on photodamage, damage prevention, and skin aging. The central hypothesis of the present study is that a daily regimen of topical ascorbic acid will lead to subjective and objective improvement in human facial photoaging and/or photodamage.

Optical profilometry is an objective method for quantification of facial wrinkles. Skin replica analysis of photodamaged skin was well described and used in previous studies of tretinoin treatment. No previous studies to our knowledge have used this technology to examine treatment with topical ascorbic acid.

RESULTS

Three patients were eliminated from the study because of inability to follow up at designated study protocol periods. Seven additional patients were excluded from analy-
sis because of breach in study protocol for active and control designations. Of the 19 evaluable subjects, 3 were male (mean age, 43 years) and 16, female (mean age, 48 years). Ages ranged from 36 to 72 years. Prestudy subject data revealed that 12 (63%) of patients had a history of smoking, 10 (52%) used sunscreens on a regular basis, and 10 (52%) admitted to excessive lifetime sun exposure. Adverse effects were mild, usually resolved within the first 2 months of therapy, and included (in decreasing order of frequency) stinging, 11 (55%); erythema, 5 (24%); and dry skin, 1 (.05%). All adverse effects were easily treated with moisturization. In no case did topical treatment required or the topical study regimen altered the mean time to first clinical improvement noted by the investigator and/or the patient was 0.7 months. Most of the initial improvement during this period involved tactile roughness and/or texture and skin hydration changes.

Table 1 summarizes the results of the computer-image analysis of skin surface impressions. Significant within-treatment changes were seen for the Ra north-south (active treatment), Rz east-west (control), Ra east-west (control), and shadows north-south (active treatment) facial axes. Tests for between-treatment differences demonstrated significantly more improvement with active treatment than with control for Ra north-south (P = .03) and shadows north-south (P = .03). The active-treatment side of the face showed more improvement in Ra north-south and shadows north-south facial axes than the control side in 14 (73.7%) of the subjects. There was a trend for more improvement in Rz north-south facial axes with active treatment than with control (P = .08). The active-treatment side showed more improvement in Rz north-south facial axes with active treatment than with control side in 13 (68.4%) of subjects.

Table 2 summarizes the results of the clinical investigator assessment. Tests for between-treatment differences demonstrated significantly more improvement with active treatment than with control for fine wrinkling (P = .002), tactile roughness (P = .04), coarse rhytids (P = .01), skin laxity/tone (P = .03), sallowness/yellowing (P = .03), and overall assessment (P = .002). In the overall assessment, 3 patients (15.8%) experienced no change on the active-treatment side of their face vs 16 (84.2%) for the control side. For the active-treatment side, 5 patients (26.3%) were slightly improved, 5 (26.3%) were improved, and 6 (31.6%) were much improved. For the control side, 3 patients (15.8%) were much improved (all others indicated no change).

Table 3 summarizes the patient questionnaire results. The test for between-treatment differences demonstrated significantly more improvement with active treatment than with control for much improved (P = .002), improved (P = .03), slightly improved (P = .03), and same (P = .002).
onstrated significantly more improvement with active treatment than with control \( (P = .002) \). The active side was preferred over the control side by 16 (84.2%) of the subjects.

Table 4 summarizes the photographic assessment results. The test for a between-treatment difference demonstrated significantly more improvement with active treatment than with vehicle control \( (P = .01) \). The active side was preferred over the control side by 11 (57.9%) of the subjects.

### Table 4. Investigator Assessment of Pretreatment and Posttreatment Photograph Comparison*

<table>
<thead>
<tr>
<th>Scoring</th>
<th>Ascorbic Acid Active Treatment</th>
<th>Vehicle Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Much improved</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Improved</td>
<td>6 (31.6)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Slightly improved</td>
<td>5 (26.3)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Same</td>
<td>8 (42.1)</td>
<td>17 (89.5)</td>
</tr>
<tr>
<td>Worse</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Data are number (percentage) of patients.

Most of the ongoing research on ascorbic acid has been directed toward the deep layers of the skin and their role in stimulating collagen synthesis and scavenging free radicals.\(^4\,5\,7\,8\,14\,15\) Ascorbate has many of the ideal properties of a free radical scavenger: excellent availability in tissues as well as adequate supply; compartmentalization in tissues; recyclability; versatility (interaction with superoxide, hydroxyl free radicals, and singlet oxygen); and tolerable toxic effects (megadoses of ascorbic acid 100 to 200 times the recommended daily allowance are generally well tolerated).\(^4\) Additionally, ascorbate can regenerate \( \alpha \)-tocopherol (the most active form of vitamin E) from the tocopherol radical, thus decreasing the vitamin E radical by means of a recycling mechanism.\(^36\,37\,38\)

Other areas of ongoing research have investigated the anti-inflammatory and wound-healing effects of ascorbic acid as well as its photopreventive effects against cutaneous malignancy. Very little information is available on the topographic changes in photodamaged facial skin associated with topical ascorbic acid application.

In this study, a 3-month daily regimen of topical ascorbic acid provided significant objective and subjective improvement in photodamaged facial skin. These changes were gradual and became progressively more evident as treatment continued. The study of topical agents for photodamaged skin is still in its infancy. Subjective clinical assessment and patient self-appraisal have been the traditional areas of evaluation. These methods demonstrated significant differences between active treatment and the control vehicle in improvement of fine wrinkling, tactile roughness, coarse rhytids, skin laxity/tone, sallowness/yellowing, and overall skin improvement. However, the limitations of this method include the obvious grading subjectivity, grading variations over time, and inconsistencies among investigator and patient assessments. Maintaining double-blindness during the study seemed difficult since most of the subjects experienced unilateral stinging during the initial 2 months of application. This may have skewed clinical and patient appraisal scores early in the study assessment by focusing on changes involving the stinging sides. Nonetheless, clinical and patient data appeared to agree that active treatment was more effective than control in most patients as the study progressed and skin texture improvement became evident. Clinical and patient self-appraisal showed an 84% correlation to predicting the active treatment side vs the control side. The vehicle control agent was matched for color, consistency, and pH to the active agent to ensure the blindness of the study. Stinging discrepancies were probably associated with variations in skin type and surface flora among subjects, as well as environmental exposures, cosmetic use, local tissue reactivity, variations in serum application, and moisturizer use.

Photographic assessment showed significant improvement, with active treatment greater than control, but was found to have the least reliability (58%) in predicting which side of the face received active treatment and which the control. Inherent limitations in photography include fluctuations in lighting, head position, and facial expression and asymmetries. Photographs represent only a moment in time and capture only a 2-dimensional image. Also, the clinical investigator knew whether the photographs were taken before or after treatment; therefore, some evaluator bias may have occurred. Furthermore, the evaluator continued to be blinded to the treatment side (active vs control). Since photographic comparisons were done at week 12 (the end of the study period), some investigator bias may have occurred secondary to knowing individual clinical and self-appraisal responses. Consequently, to complement subjective evaluations, skin replica optical profilometry was used to quantify changes in skin surface texture with minimal variability and potential for bias. Other researchers have used this technology in various forms of cutaneous clinical research and have documented its validity as an objective measure of skin topography.\(^31\,33\)

In this study, skin replica data corroborated clinical findings in 14 patients (74%). These results are similar to those of other studies that have shown a significant degree of correlation between clinical grade and image analysis measurements.\(^31\,33\) Replicas matched active treatment and control in 15 patients (79%).

No significant differences were seen in the east-west orientation for any of the parameters. Significant improvement was noted in the pretreatment and post-treatment comparisons in the north-south axis where the illumination runs across the major facial lines and highlights them. Pretreatment and post-treatment comparison revealed significant improvement with active treatment greater than control for \( R_x \) north-south facial axes \( (P = .03) \) and shadows north-south facial axes \( (P = .03) \). In the case of \( R_z \) north-south axes, the values approached significance \( (P = .08) \). Therefore, the overall topography of skin treated with active topical ascorbic acid is smoother and less wrinkled than that treated with control. The fact that \( R_z \) and shadows represent fine
to intermediate-depth lines and that $R_z$ is largely a measure of deep wrinkles suggests that topical ascorbic acid therapy had a more dramatic effect on superficial topography than on major furrows and creases. These findings are supported by the clinical investigative data showing more significant improvement in fine wrinkling than in coarse rhytids (though rhytids were still significantly improved). The use of computer image analysis of skin surface replicas added a dimension of objectivity to the evaluation, but its use is technique dependent. One must be astute to detail and consistency in patient positioning, site preparation and cleansing, precise application and reliable relocation of adhesive rings, proper mixture, and timing of application of silicone replica material. Despite these shortcomings, it was found that with experience and repetition, one can achieve consistent results.

In a hemiface trial, there may be some crossover of active treatment to the control side of the face and vice versa. Steven Mandy, MD, of the Aspen Skin Center, Aspen, Colo, has compiled unpublished data that may call into question the validity of hemiface trials using topical creams (personal communication, May 1998). Mandy used fluorescein dye in tretinoin creams in hemiface studies and found, under UV light, evidence of “creep” (the study cream melted and flowed onto the control side). However, both the amount and incidence of this crossover was not significant. Also, one must be aware that, in hemiface trials, crossover hand application contamination may occur. In this study, patients were given verbal instructions on crossover prevention; they were told to wash their hands between the application of the active treatment and the vehicle control and to use an alternate-hand application technique. However, one cannot completely control and/or measure patient compliance in these matters.

It should also be mentioned that there is an increasing number of ascorbic acid–based topical cosmetics available on the market, and it seems that not all preparations of topical ascorbic acid are effective. Many of these products use derivatives, esters, and analogs of ascorbic acid that do not penetrate the skin, do not chemically convert to L-ascorbic acid (the only form that can be used by the body), and/or are not delivered in adequate concentration. These ascorbic acid substitutes include ascorbyl palmitate, magnesium ascorbyl phosphate, ascorbic acid sulfate, ascorbyl stearate, ascorbyl dipalmitate, and ascorbic acid magnesium phosphate, which are easily stabilized but must be converted to L-ascorbate to be effectively useful. There is no direct evidence that ascorbic acid derivatives enter the skin in appreciable amounts, and it seems that their conversion to L-ascorbate is largely inefficient, thus precluding effective concentration delivery.

This 3-month study demonstrated and evaluated topographic improvement in photodamaged facial skin treated with topical L-ascorbic acid, tyrosine, and zinc (Cellex-C).

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The author (Dr Traikovich) and fellowship director (William H. Beeson, MD) deny any financial relationship and/or obligation to the manufacturers of the pharmaceutical and technological products used in this study. Dr Traikovich has received no direct or indirect compensation of any kind. Neither he nor any member of his family has financial interest in the products or companies named in this article. Results of this study have not and will not be communicated to Cellex-C International, Toronto, Ontario, or any of its vendors or agents. The sole purpose of this study was to fulfill requirements of the American Association of Facial Plastic and Reconstructive Surgery fellowship and is not to be used as promotional or marketing material for any pharmaceutical concern.

Cellex-C International provided free Cellex-C for 1 year to those subjects who completed the 3-month trial. For the second year, the subjects who completed the trial were able to purchase the product for half price. Cellex-C International provided all active and control serum used in this study at no cost or obligation to the Beeson Aesthetic Surgery Institute.

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