

Double-Blind, Half-Face Study Comparing Topical Vitamin C and Vehicle for Rejuvenation of Photodamage

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BACKGROUND. Aging of the population, in particular the “baby boomers,” has resulted in increased interest in methods of reversal of photodamage. Non-invasive treatments are in high demand, and our knowledge of mechanisms of photodamage to skin, protection of the skin, and repair of photodamage are becoming more sophisticated and complex.

OBJECTIVE. The objective of this study is to determine if the topical use of a vitamin C preparation can stimulate the skin to repair photodamage and result in clinically visible differences, as well as microscopically visible improvement.

METHODS. Ten patients applied in a double-blind manner a newly formulated vitamin C complex having 10% ascorbic acid (water soluble) and 7% tetrahexyldecyl ascorbate (lipid soluble) in an anhydrous polysilicone gel base to one-half of the face and the inactive polysilicone gel base to the opposite side. Clinical evaluation of wrinkling, pigmentation, inflammation, and hydration was performed prior to the study and at weeks 4, 8, and 12. Two mm punch biopsies of the lateral cheeks were performed at 12 weeks in four patients and stained with hema-

toxylin and eosin, as well as in situ hybridization studies using an anti-sense probe for mRNA for type I collagen. A questionnaire was also completed by each patient.

RESULTS. A statistically significant improvement of the vitamin C-treated side was seen in the decreased photoaging scores of the cheeks ($P = 0.006$) and the peri-oral area ($P = 0.01$). The peri-orbital area improved bilaterally, probably indicating improved hydration. The overall facial improvement of the vitamin C side was statistically significant ($P = 0.01$). Biopsies showed increased Grenz zone collagen, as well as increased staining for mRNA for type I collagen. No patients were found to have any evidence of inflammation. Hydration was improved bilaterally. Four patients felt that the vitamin C-treated side improved unilaterally. No patient felt the placebo side showed unilateral improvement.

CONCLUSION. This formulation of vitamin C results in clinically visible and statistically significant improvement in wrinkling when used topically for 12 weeks. This clinical improvement correlates with biopsy evidence of new collagen formation.

L-ASCORBIC ACID, commonly known as vitamin C, is an essential requirement and nutrient for humans. However, humans do not have the ability to synthesize ascorbate from glucose because the final enzyme in ascorbate synthesis is not present.¹ Vitamin C must be ingested from citrus fruits and dark-green leafy vegetables or applied topically to the skin in various preparations in order to be present as an essential factor for life and for skin protection. Vitamin C is necessary for the normal formation and maintenance of collagen. Indeed, scurvy is the classic manifestation of severe ascorbic acid deficiency. Its symptoms are due to loss of the cementing action of collagen.²⁻⁴ Not only is ascorbic acid necessary as a cofactor for several hydroxylating enzymes,⁵⁻⁷ resulting in maximum stability of the collagen triple helix and secretion of procollagen from the cell, as well as participation in cross-

linking of fibers, but it may also influence collagen synthesis independent of hydroxylation.^{8,9} Studies have demonstrated stimulation of collagen synthesis by specifically increasing the levels of mRNA for three different pro- α chains.⁸ Collagen production has been demonstrated to be stimulated by proline hydroxylation as well.^{10,11}

Ascorbic acid is known to be a powerful antioxidant and is the primary water soluble nonenzymatic biological antioxidant in human tissues.^{1,12-15} It should be noted, however, that though water soluble antioxidants act as a first defense, particularly against radicals generated in plasma, they cannot scavenge lipophilic radicals within the membranes.¹⁶ Lipophilic chain-breaking antioxidants, particularly vitamin E, suppress oxidative damage efficiently in membranes.¹⁶ In addition to its direct antioxidant effects, ascorbic acid is known to be the primary replenisher of vitamin E, the most efficient inhibitor of lipid peroxidation.¹⁷⁻²¹

It is now well accepted that oxygen free radicals are involved in cutaneous photodamage.²²⁻²⁵ Studies show that long-wave ultraviolet A (UVA) (340–400 nm) alone can cause photoaging of the skin^{26,27} and that reactive oxygen species trigger these changes.²⁸ It has been

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demonstrated that the skin's native antioxidant protection breaks down during this UV assault.²⁶⁻²⁸

As a consequence of the knowledge of vitamin C's activities as an antioxidant and stimulant of collagen production, its use topically has been suggested, as oral administration is thought to be incapable of generating adequate tissue levels of ascorbic acid for these tissue effects.²⁹

Topical delivery of ascorbic acid is complicated by its instability (an advantage as an antioxidant) and its water solubility in penetration of the lipophilic stratum corneum. The use of lipid soluble, pH neutral, nonirritating esters has been suggested as a solution to these problems,³⁰ as well as using ascorbic acid in a preparation with a pH below ascorbate's first pK_a in its nonionic form.³¹ Topical application of vitamin C has been shown to provide photoprotection^{28,30,32,33} to decrease radiation damage³⁴ and to have an anti-inflammatory effect in psoriasis.³⁰ Photoprotection may occur in the stratum corneum without penetration into the epidermis or the dermis.²⁸ However, to prevent photodamage to collagen and elastin and to stimulate collagen synthesis, vitamin C must penetrate to the dermis. No studies have been performed documenting clinical evidence of new collagen production secondary to topical application of vitamin C in patients having facial photodamage. Application of vitamin C to a specific site where connective tissue formation is needed may alleviate the conditions of evidential aging such as wrinkling or actinic aging, caused by exposure of the skin to ultraviolet radiation.

It would be useful to have a composition for topical application that has enhanced stability and bioavailability characteristics. A polysilicone gel was devised such that the preparation was anhydrous and the L-ascorbic acid was suspended within the gel. Also, the use of an anhydrous vehicle allowed for time-released delivery of the vitamin C without the usual rapid degradation seen with other preparations. In addition, the use of a new active lipid form of pro-vitamin C, namely tetrahexyldecyl ascorbate, was utilized for its increased ability to penetrate and its ultimate enzymatic conversion to vitamin C within the dermis. This form of vitamin C also demonstrates superior stability to ascorbic acid used in other aqueous preparations, further ensuring the delivery of vitamin C to the dermis and the fibroblasts.³⁵ An investigation of the topical effects of such a preparation follows.

Materials and Methods

Ten patients having facial photodamage were recruited for a double-blind pilot study of a newly formulated vitamin C complex having 10% ascorbic acid, a water soluble acid, and 7% tetrahexyldecyl ascorbate, a lipid soluble analog.

Both of these are combined in an anhydrous polysilicone gel base, which acts as a "dermal patch," releasing the water soluble acid slowly and the lipid soluble analog rapidly. The active vitamin C complex was applied to one side of the face and the inactive placebo base was applied to the opposite side of the face once a day.

Prior to treatment the patients' skin hydration, dyspigmentation, and wrinkling were assessed by the examining physician, that being one of three different physicians at each grading session. Each side was assessed separately. The assessment of hydration was on a scale of 1-3, with 2 being normal and 1 being dry. The dyspigmentation was graded on a scale of 1-4, with 1 being none and 4 being severe. Wrinkling was graded in 0.5 increments on a scale of 1-9, as previously reported,³⁶ with 1 being minimal and 9 being most severe. Inflammation of the skin was also assessed as present or absent. Assessments of each side were repeated at weeks 4, 8, and 12. Biopsies of both sides of the face, sampling a treated area as well as a control area, were performed at week 12 in four patients. These biopsies were performed as 2 mm punches anterior to the ear on the lateral cheek. There was no attempt to select an area of apparent clinical improvement. Biopsies were stained with hematoxylin and eosin and read at 10 \times power. Three measurements of Grenz zone collagen and epidermal thickness per section for four sections of each specimen were performed with an in-lens reticle. This same protocol of measurement was repeated 1 month later and the two groups of measurements averaged as the reported reading. Measurements were taken in units of 15 μ m, starting from the surface of the epidermis to the dermoepidermal junction and from the superior aspect of pink-staining papillary dermal collagen underlying the epidermis to the gray-blue staining of the solar elastosis, the so-called Grenz zone. The presence or absence of an inflammatory infiltrate was noted as well. A comparison of left side to right side regarding improvement of hydration, texture, pigmentation, and wrinkling over the 12 weeks of the study was completed by the patients.

Statistical analysis was performed using the paired *t*-test. Statistical significance was defined as $P < .05$.

Results

Wrinkling Scores

In the periorbital area, the average pretreatment scores of the treatment side and gel-base side were 6.45 and 6.20, respectively. The average 12-week scores were 5.25 and 5.30, respectively, revealing an average improvement of 18.6% on the treatment side ($P = .0005$) (Figure 1) and 14.5% ($P = .001$) on the placebo side (Table 1). On the treatment side, 8 of 10 patients improved at least one point in severity grading of the wrinkles (Table 2). The corresponding gel-base side showed a greater than one point improvement in three of these eight patients. On the gel-base side, 5 of 10 patients showed at least a one point improvement

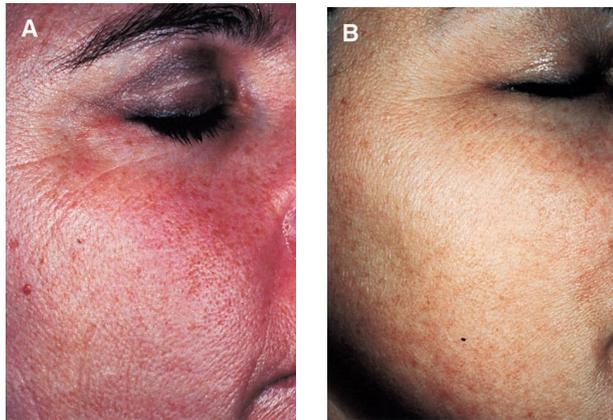


Figure 1. A) Significant facial photodamage with textural irregularity as well as deep lines prior to therapy. B) Visible improvement in texture and lines is seen after 90 days of topical vitamin C application.

and 3 of these 5 had similar improvement on the treatment side.

In the perioral area, the average pretreatment score of the active agent side was 6.35 and that of the gel-base group was 6.10. After 12 weeks the active side score was 5.40, a 15.0% improvement ($P = .01$) and the gel base side was 5.95, a 2.5% improvement ($P = .17$). Six of 10 patients improved at least one point on the active side, while 1 of these 6 showed similar improvement on the gel-base side. Only 2 of 10 patients showed a one point improvement on the gel-base side and 1 of the 2 patients had similar improvement on the treatment side.

In the cheek area, the pretreatment score of the active agent side was 6.05 and that of the gel-base side was 5.70. After 12 weeks the active agent side had improved to 5.25, an improvement of 13.0% ($P = .006$), while the gel-base side improved 4% to 5.45 ($P = .22$). On the active agent side, 4 of 10 patients showed at least a one point improvement and none of those 4 patients showed a similar improvement on the gel-base side. One of 10 patients showed a one point improvement on the gel-base side. There was no improvement on the treatment side in this patient.

In the forehead area there were three patients who showed a greater than one point improvement on the

Table 2. Topical Vitamin C: Improvement in Wrinkle Scores >1 Point

	Vitamin C complex	Vehicle
Periorbital	8	5
Forehead	3	2
Cheeks	4	1
Perioral	6	2

treatment side and two of these three patients showed improvement on the gel-base side. The pretreatment scores of the treatment side averaged 5.70 and improved to 5.25, an improvement of 8.0% ($P = .07$). Three of 10 patients showed at least a one point improvement on the gel-base side, with 2 of these 3 showing similar improvement on the gel-base side. The pretreatment score averaged 5.70 and the posttreatment score improved to 5.30, a 7% change ($P = .15$).

On the treatment side, 4 of 10 patients showed improvement in all areas except the forehead (Table 3). None of these patients showed similar improvement on the gel-base side. Excluding the forehead area, the average score of the other three areas before treatment was 6.38 on the treatment side and 6.00 on the gel-base side. The average improvement on the treatment side was 25%, reflecting a score of 4.79 after treatment ($P = .01$) (Table 4). By comparison, the gel-base side had a starting score of 6.00, ending score of 5.54, and an improvement of 7.67% or 0.46 ($P = .08$) (Table 5).

Hydration, Pigment, and Inflammation

In the physicians' evaluation, 4 of 10 patients had normal hydration at the beginning of the study and were unchanged at 12 weeks. The six remaining patients were graded as having drier than normal skin at the beginning of the study and all improved to normal hydration at the 12-week evaluation. All hydration evaluations were equal bilaterally. Five patients were judged to have moderate or severe dyspigmentation at the start of the study. Three of these patients improved on the treatment side and three improved on the gel-base side. Two of these patients showed bilateral improvement. No patients were found to have either clinical or histologic evidence of inflammation on either side of the face.

Table 1. Topical Vitamin C: Average Improvement in Wrinkle Scores

	Vitamin C complex	Vehicle
Periorbital	18.6%	14.5%
Forehead	8.0%	7.0%
Cheeks	13.0%	4.0%
Perioral	15.0%	2.5%

Table 3. Topical Vitamin C: Patients with >1 point Improvement in Periorbital, Cheek, and Perioral Areas

	No.	Average Score		Average Improvement
		Before	After	
Vitamin C complex	4	6.38	4.79	25%
Vehicle	0	6.00	5.54	8%

Table 4. Topical Vitamin C for Reversal of Photodamage

	Vitamin C Pre	Vitamin C Post
Periorbital	6.45	5.25
Perioral	6.35	5.4
Cheeks	6.05	5.25
Forehead	5.7	5.25
Significance	P = 0.01	

Patients' Evaluation

The patients' evaluation at 12 weeks was a comparison of the left side to the right side regarding differential improvement in wrinkling, hydration, and pigment. Four patients felt that on the side that received active agent, wrinkles improved more than the side receiving gel base. Three of these four patients were in the group of four patients who were judged by the physicians to have improved in all three areas other than the forehead on the same treatment side. No patients rated the improvement in wrinkles of the gel-base side as being greater than that of the active agent side.

Three patients thought that the skin hydration of the active agent side improved more than the gel-base side, while one patient felt the gel base side improved more. No patients felt that there was a differential improvement in pigment.

Biopsies

The average epidermal thickness of the treatment side was 51.8 μm , while that of the gel-base side was 48.1 μm . The Grenz zone collagen measurements averaged 52.5 μm on the treatment side and 37.5 μm on the gel-base side. There was one patient that showed no difference between the treatment and gel-base sides, while three patients showed an increase on the treatment side. In situ hybridization studies using an antisense probe for mRNA for type I collagen revealed more intense staining on the vitamin C side than the gel-base side (4+ versus 3+) in three of the four patients studied. The one patient showing no difference in staining was the same patient showing no difference in Grenz zone collagen (Figure 2).

Table 5. Topical Vitamin C for Reversal of Photodamage

	Placebo Pre	Placebo Post
Periorbital	6.2	5.3
Perioral	6.1	5.95
Cheeks	5.7	5.45
Forehead	5.7	5.3
Significance	P = 0.08	

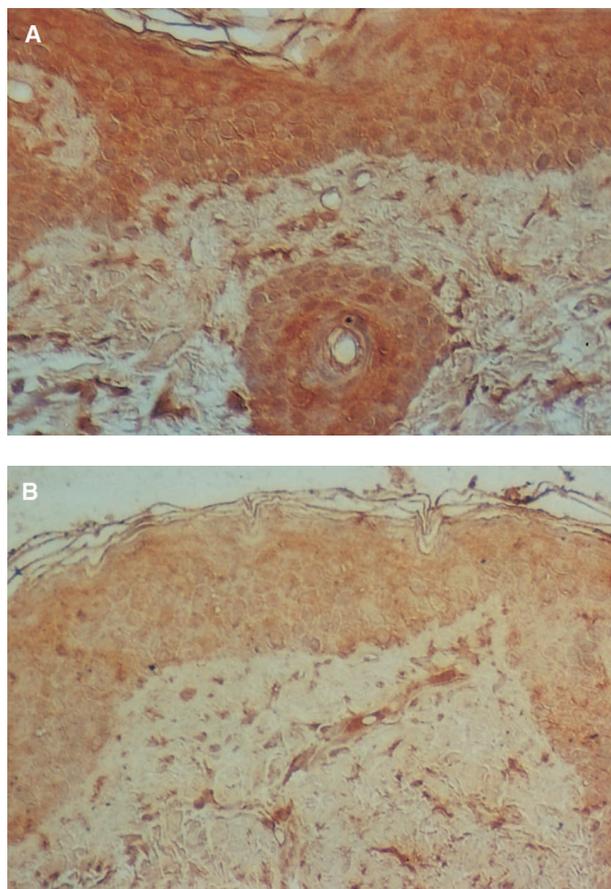


Figure 2. A) Immunohistologic staining for mRNA for collagen type I in a biopsy of the topical vitamin C-treated side. B) Less visible staining is seen on the placebo side.

Discussion

Treatment of photodamage with topical preparations of vitamin C has become very popular over the past several years, based on animal studies demonstrating the ability to block UV-induced erythema by topical application of vitamin C preparations, as well as in vitro studies demonstrating stimulation of collagen production by fibroblasts.

However, there are few, if any, clinical studies demonstrating a clinical benefit in reversal of photodamage by topical application of vitamin C or its analogs. Indeed, there are studies showing penetration of ascorbic acid into the dermis when the pH is less than 3.0. There are also patents and studies demonstrating dermal penetration of lipid soluble analogs. Arguments have been made for the use of water soluble ascorbic acid in order to have adequate intracellular content versus those who argue for lipid soluble analogs in favor of localization to cell membranes for protection of cell membrane integrity. Problems exist with low pH ascorbic acid formulations—they are unstable in cos-

metic preparations and burn or irritate skin upon application, thereby countering anti-inflammatory benefits. Ascorbic acid analogs have a less irritating effect, but less proven conversion to active forms in the skin.³⁷

The current study demonstrates definite clinical benefit from the use of a combination of anhydrous ascorbic acid and lipid soluble tetrahexyldecyl ascorbate. It is our theory that water soluble ascorbic acid is slowly released into the stratum corneum and acts primarily as an antioxidant in protecting the skin, while the lipid soluble tetrahexyldecyl ascorbate penetrates directly into the epidermis and dermis, acting both as an antioxidant and a direct stimulant of new collagen. Studies by Barnet Products Corporation³⁵ have shown that lipid soluble tetrahexyldecyl ascorbate not only penetrates through the epidermis better than ascorbic acid, but to the dermis as well. Penetration rates are dose dependent, but tetrahexyldecyl ascorbate surpasses the penetration of ascorbic acid by threefold at the same concentration and maintains a higher penetration rate even when ascorbic acid concentration is 25 times that of tetrahexyldecyl ascorbate.

Statistically significant differences in clinical scoring of facial wrinkles at the end of the 90-day study were detected in the perioral and cheek areas in the 10 patients studied when the two sides were compared. There was also statistically significant improvement of the entire treated side compared to its beginning point and no significant change on the base side. It is interesting that hydration improved bilaterally in all patients with dry skin preoperatively and that the lines of the forehead and periorbital areas improved bilaterally in the placebo-treated and vitamin C-treated sides, indicating that the improvement seen in these areas is more likely related to enhanced hydration of the skin. These hydration improvements resulted in statistically significant improvement bilaterally compared to the starting condition.

Biopsies of treated versus untreated areas confirmed the presence of increased amounts of collagen in those patients showing clinical improvement. In addition, there was good correlation of the independent judgment of the patients and that of the treating physicians in identifying the areas of improvement in a double-blind study. Studies by Barnet Products Corporation³⁵ have shown that in fibroblast cultures, 50 μm of tetrahexyldecyl ascorbate doubles collagen synthesis, while the same dosage of ascorbate acid increases collagen synthesis by only 25%.

There was no identifiable difference in pigmentation of the treated versus the untreated side, though some studies have shown lightening of lentigines, ephelides, and melasma. Tetrahexyldecyl ascorbate has been shown to reduce melanogenesis by more than 80% in human melanoma cell cultures.³⁶

This pilot study has demonstrated that properly formulated vitamin C topical products may play a significant role in the reversal of photodamage of the skin. This type of product may be used as part of an overall program using other topical agents with or without other procedures such as various ablative and nonablative laser therapies, acid peel resurfacing, or dermabrasion.

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