The Effects of Filtrate of the Secretion of the *Cryptomphalus aspersa* on Photoaged Skin

Sabrina Guillen Fabi MD,[a] Joel L. Cohen MD,[b] Jennifer D. Peterson MD,[c] Monika G. Kiripolsky MD,[d] and Mitchel P. Goldman MD[a,e]

[a]Goldman, Butterwick, Fitzpatrick, Groff, & Fabi, Cosmetic Laser Dermatology, San Diego, CA
[b]AboutSkin Dermatology and DermSurgery, Englewood, CO
[c]Dermatology Laser Surgery, Houston, TX
[d]San Diego Skin & Laser, Carlsbad, CA
[e]Department of Dermatology/Medicine, University of California, San Diego, San Diego, CA

ABSTRACT

Background: Growth factors (GFs) are chemical messengers that regulate specific cellular activities such as cell proliferation and formation of the extracellular matrix. GFs may be derived from a variety of sources, including animals.
Objective: Evaluate the safety and efficacy of a topical antiphotoaging product containing secretions of the snail *Cryptomphalus aspersa* (SCA) for the improvement of facial rhytides.
Materials and Methods: This was a 2-center, double-blind, randomized, 14-week study in which 25 patients with moderate to severe facial photodamage were treated with an emulsion (with 8% SCA) and liquid serum (with 40% SCA) on one side of the face and placebo on the contralateral side for 12 weeks. Silicone skin impressions of periocular rhytides were performed at baseline and after 12 weeks of treatment. Patient and physician assessments were also performed at 8, 12, and 14 weeks.
Results: Periocular rhytides on the active ingredient side showed significant improvement after 12 weeks (P=.03) and improved texture to a greater degree than placebo at 8 and 12 weeks, as well as 2 weeks after discontinuing the product (14 weeks).
Conclusion: Daily application of topical products containing SCA proved effective and well tolerated for improvement in coarse periocular rhytides and fine facial rhytides. Subjects noted a significant degree of improvement in fines lines at the 8-week time point on the SCA-treated side ($P \leq .05$) but did not report a significant difference in the quality of their skin.

J Drugs Dermatol. 2013;12(4):453-457.

INTRODUCTION

Skin's mechanistic, protective, and restorative properties decline with age. Daily exposure to environmental stressors, including sunlight and pollution, accentuate the skin's underlying inherent deterioration related to chronological aging. "Aged" skin (whether chronologically or photorelated) manifests as xerosis, loss of elasticity, atrophy, dyschromia, as well as fine and deep rhytides. The search for safe, noninvasive treatments to reverse these pathophysiologic changes associated with aging skin remains challenging.

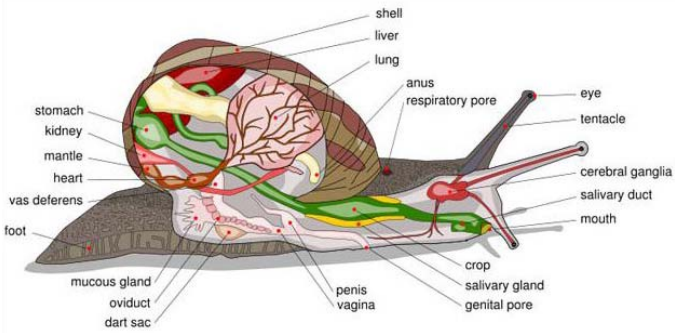
Growth factors (GFs) are chemical messengers that regulate specific cellular activities such as cell proliferation, chemotaxis, and formation of the extracellular matrix.[1] They may be derived from a variety of sources, including humans, animals, microbes, as well as yeast and plants.[2] Topical GFs have emerged as a therapeutic modality harnessed for aesthetic and medical uses. As our understanding of the mechanisms of action behind these powerful GF compounds increases, so does our ability to fully apply the benefits associated with these mechanisms in the clinical setting.

While there is documentation that GFs derived from human sources provide some benefit in wound healing and repair of photodamage, there are also concerns of potential deleterious side effects, including tumorigenesis.[3-5] Because of these concerns, scientists have long considered nonhuman sources for GFs. One successfully yoked *animal*-derived GF, the secretion of the snail *Cryptomphalus aspersa* (SCA), was discovered by Rafael Abad Iglesias MD, a radiation oncologist treating radiation dermatitis.[6] It was noted that several species of mollusk retract their tentacles when exposed to ultraviolet (UV) light and x-rays. When this defense mechanism was further explored, a biologically active glycosaminoglycan secretion was found to be generated by the snail during times of stress. The secretion is composed of a combination of contributions from the snail's mucous, salivary, and proteic glands. SCA stimulates biochemical, structural, and functional processes and can regenerate damaged structures of the animal's skin in less than 48 hours. Figure 1 reveals SCA-related anatomy.

SCA has since been processed into a topical product (Tensage; Biopelle, Inc, Ferndale, MI, manufactured by Industrial Farmaceutica Cantabria, SA) with proclaimed antiphotoaging effects. Through a patented process, snails are stimulated and their secretions are collected. These secretions are then filtered for purity and tested for consistency. Of note, snails are not harmed during this process, considering that secretions produced during snail

death (ie, from overexertion) are thought to be contaminants of the therapeutic SCA, thus further reason to avoid harming the snails during the collection of the secretions.

FIGURE 1. The anatomy of the snail Cryptomphalus aspersa.



MATERIALS AND METHODS

The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki and was approved by the independent institutional review board. All patients consented to participation in the study and were provided a copy of the informed consent.

Materials

The 8% SCA emulsion (Tensage Contour Cream; Biopelle, Inc) used in this study contains water, snail secretion filtrate, C12-20 acid PEG-8 ester, C12-15 alkyl benzoate, ethoxydiglycol, glycerin, saccharide isomerate, hexylene glycol, PEG/PPG-20/6 dimethicone, fructose, glucose, phenoxyethanol, tocopheryl acetate, cetyl alcohol, methylparaben, propylparaben, dextrin, sucrose, urea, disodium EDTA, sodium citrate, tetrahydrodiferuloylmethane, alanine, aspartic acid, glutamic acid, hexyl nicotinate, and fragrance. The 40% liquid serum (Tensage Intensive Ampoules; Biopelle, Inc) used in this study contains snail secretion filtrate, propylene glycol, water, saccharide Isomerate, hexylene glycol, polysorbate 20, PEG/PPG-20/6 dimethicone, sodium ascorbyl phosphate, sodium chloride, fructose, glucose, polyquaternium-10, tocopheryl acetate, citric acid, tetrasodium EDTA, dextrin, sucrose, urea, alanine, aspartic acid, glutamic acid, hexyl nicotinate, and fragrance. The placebo emulsion used in this study contained water, C12-20 acid PEG-8 ester, C12-15 alkyl benzoate, ethoxydiglycol, glycerin, saccharide isomerate, hexylene glycol, PEG/PPG-20/6 dimethicone, fructose, glucose, phenoxyethanol, tocopheryl acetate, cetyl alcohol, methylparaben, propylparaben, dextrin, sucrose, urea, disodium EDTA, sodium citrate, tetrahydrodiferuloylmethane, alanine, aspartic acid, glutamic acid, hexyl nicotinate, and fragrance. The placebo serum used in this study contained propylene glycol, water, saccharide isomerate, hexylene glycol, polysorbate 20, PEG/PPG-20/6 dimethicone, sodium ascorbyl phosphate, sodium chloride, fructose, glucose, polyquaternium-10, tocopheryl acetate, citric acid, tetrasodium EDTA, dextrin, sucrose, urea, alanine, aspartic acid, glutamic acid, hexyl nicotinate, and fragrance.

Study Population

Females aged 35 to 65 years with Fitzpatrick skin types II and III demonstrating periocular and perioral wrinkling, corresponding to a grade 3 or higher on the Rao-Goldman's 5-point wrinkle evaluation scale (RGWS) were eligible for enrollment. Thirteen subjects were enrolled at one site, and 12 subjects were enrolled in the second study site. Exclusion criteria included the following: unwillingness to avoid excessive sunlight or wear protective clothing and sunscreen, unwillingness to forgo any other topical dermatological or drug therapy (including corticosteroids) on the face, as well as use of both α- and β-hydroxy acids, retinoids, or vitamin C– or D–containing topicals within 30 days before as well as throughout the course of the study.

Washout periods adhered to by subjects in this study included the following: 6 months free from dermabrasion, deep chemical peels, ablative laser treatments, neurotoxin or filler injections, and cosmetic surgery; 3 months free from nonablative laser, light (including intense pulsed light), or radiofrequency treatments; and 1 month free from microdermabrasion as well as light- and medium-depth chemical peels.

Methods

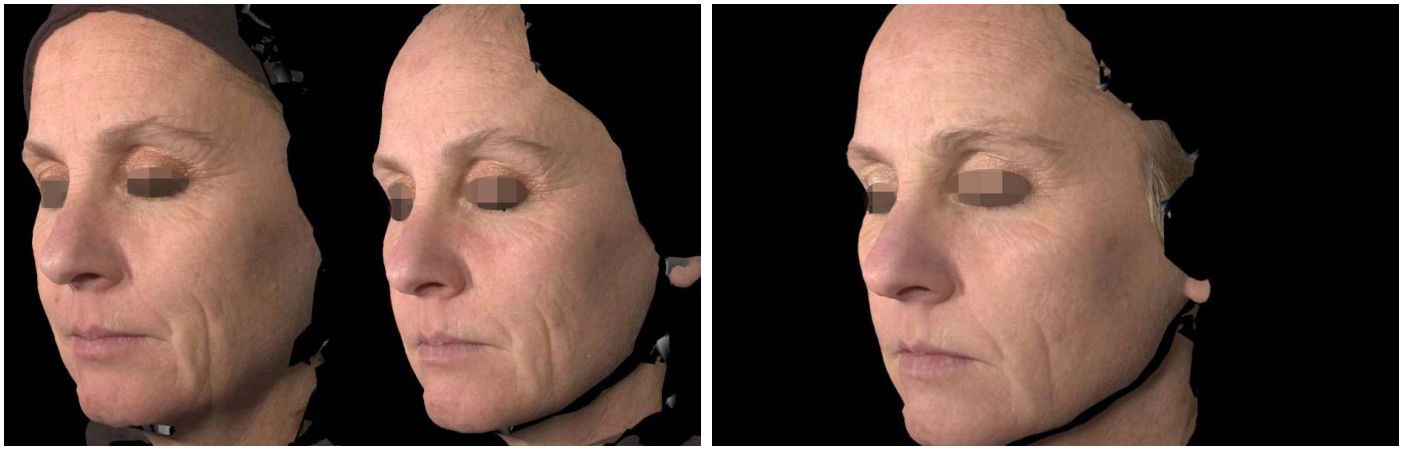
A 2-center, double-blind, placebo-controlled, randomized study to evaluate the safety and efficacy of a daily skin care regimen consisting of an 8% SCA emulsion (Tensage Contour Cream; Biopelle, Inc), available as a 15-g eye contour cream, and a 40% liquid serum (Tensage Intensive Ampoules, Biopelle, Inc), manufactured in a box of 10 ampoules, compared with that of an inactive control emulsion and liquid (corresponding to the active SCA vehicle) in patients with moderate to severe photodamage was performed.

Each subject's treatment regimen included daily morning use of a bland cleanser followed by randomized, double-blind, split-face application of SCA 8% emulsion, with the contralateral side receiving an inactive control emulsion. Sunscreen was applied as well. The evening regimen included use of a bland cleanser followed by randomized, double-blind, split-face application of SCA 40% serum, with the contralateral side receiving an inactive control serum. This regimen was maintained for 12 weeks.

In addition to subjective patient evaluations of facial skin and adverse events, investigator assessments and 3-D imaging were conducted at baseline (day 0) as well as at weeks 8 and 12. Subjects rated their improvement of skin quality at weeks 8 and 12. Additional investigator assessments were performed at week 14, which was 2 weeks after discontinuation of product use.

The severity/depth and number of periocular rhytides on each side of the face was assessed through silastic skin impressions using the Repliflo system (Cuderm Corporation, Dallas, TX). A dispensing instrument was loaded with the Repliflo cartridges and mixing tips appropriately assembled in the instrument. The

FIGURE 2. Active side Caucasian female at baseline (left) and 12 weeks (right). Photos courtesy of Mitchel P. Goldman MD.



resin and catalyst are squeezed in equal proportions into the mixing tube, becoming a single material. Continuous pressure ejects a thin layer of the material to the skin inside an adhesive ring attached to the periocular canthus. The material was allowed to set for at least 5 minutes before the ring was removed. The replica was inspected for defects and only accepted if no visible defects were present. The replica was allowed to dry for 24 hours and stored in a protective case until analysis. Replicas were performed at baseline as well as after 12 weeks of treatment.

The replica was analyzed by placing it in a mount that fixed the direction of the tab position of the replica so that the replica could be rotated to align the tab direction normal or parallel to the incident light direction. The replicas were illuminated with a collimated light source directed at a 25° angle from the plane of the replica. The normal sampling orientation provides texture measurements sensitive to the major, expression-induced lines (crow's feet), whereas the parallel sampling orientation provides texture measurements sensitive to the minor, fine lines. Surface texture was measured by the luminance along a set of 10 equal length parallel lines (passes) running across the replica parallel to the lighting direction. The variations in luminance were treated as indicative of the roughness and analyzed by traditional surface roughness calculations. The measurement is the brightness profile generated by the angled lighting of the wrinkles on the replica. Note that the amplitude of the profile is not proportional to the depth of the wrinkle, but represents the intensity of the *shadows* behind the wrinkles and *highlights* in front of the wrinkles.

The primary end point was the assessment of changes in both perioral and periocular rhytides as measured by the 5-point RGWS (1 = wrinkles absent, 2 = shallow but visible wrinkles, 3 = moderately deep wrinkles, 4 = deep wrinkles with well-defined edges, 5 = very deep wrinkles with redundant folds). The secondary end point was to measure changes in texture and photodamage severity of both perioral and periocular skin on a 4-point texture scale (1 = smooth and soft, 2 = slightly coarse and grainy, 3 = coarse and grainy, 4 = bumpy and uneven), and 4-point Glogau scale, respectively. Patient assessments of fine lines, tightness, texture, hydration, dyschromia, luster, and satisfaction for facial hemispheres treated with SCA products vs those treated with inactive preparations were also tabulated.

Data analysis was performed using the paired *t* test. All tests were 2-sided, and a *P* value of ≤.05 was considered statistically significant.

RESULTS

Twenty-five female subjects were enrolled in this study. The mean age of the patients enrolled was 55.6 years (range, 45-65 years). One subject was lost to follow-up and thus was not included in the final data analysis. The mean periocular and perioral rhytid severity scores, as measured by the RGWS, were 3.69 and 3.46 at baseline, respectively. The severity of periocular rhytides improved by 0.6 points on the RGWS on the SCA-treated side by week 12 (P=.03), where no statistically significant difference was seen on the placebo-treated side (Figure 2). At weeks 8, 12, and 14, the SCA-treated side showed a greater difference in improvement in skin texture of both the periocular and perioral areas when compared with placebo (Figures 3 and 4). No change in overall photodamage severity, as measured by the Glogau scale, was appreciated between the different treated sides at any of the visits.

Between baseline and any of the follow-up visits, there was no significant difference between patient assessments on quality of facial skin (using a 4-point scale), including dryness, oiliness, texture, lines, or wrinkles around both the eye and mouth area, tightness, pigmentation, skin thickness, and overall satisfaction with facial skin properties. When subjects were asked to rate their degree of improvement in fine lines, skin elasticity or tightness, texture, hydration, and global skin appearance from baseline at visit 8 and 12, a significant degree of improvement was noted in fines lines at the 8-week time point on the SCA-treated side (P≤.05).

FIGURE 3. Active side of a Caucasian female at baseline (left) and 12 weeks (right) with periocular and perioral areas highlighted. Active-treated side at baseline with less severe photodamage as compared with right side. Photos courtesy of Mitchel P. Goldman MD.

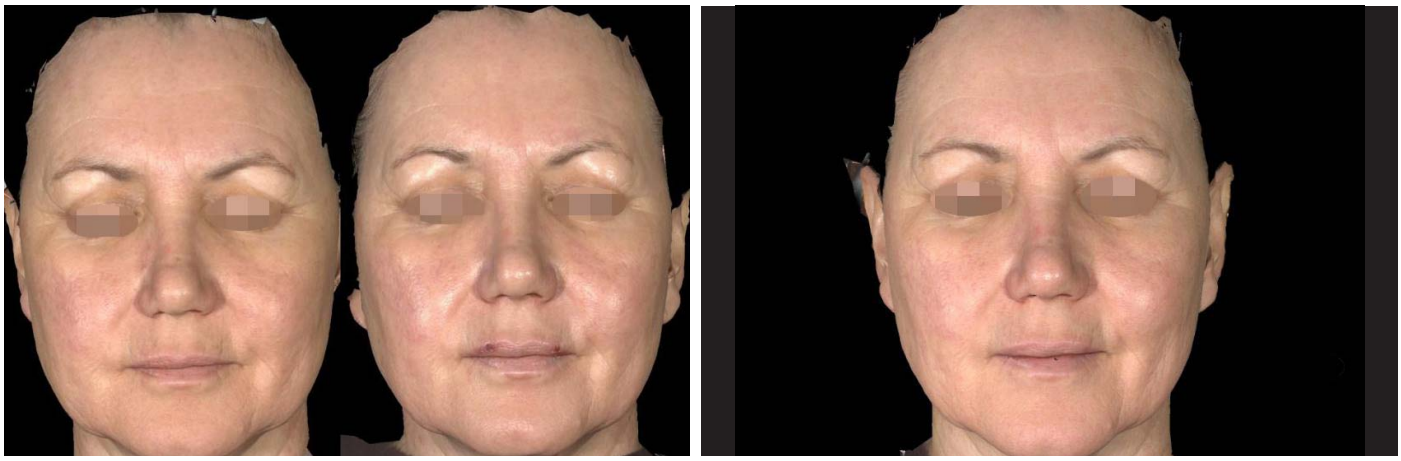


FIGURE 4. Caucasian female at baseline (left) and 12 weeks (right). Left side of the face is active. Photos courtesy of Joel L. Cohen MD.

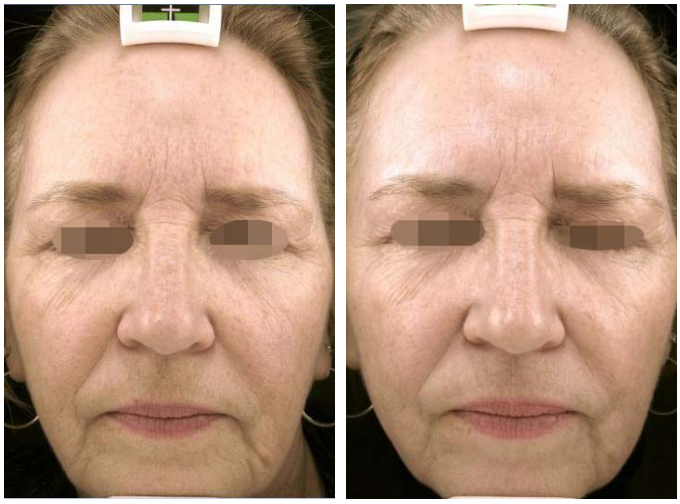
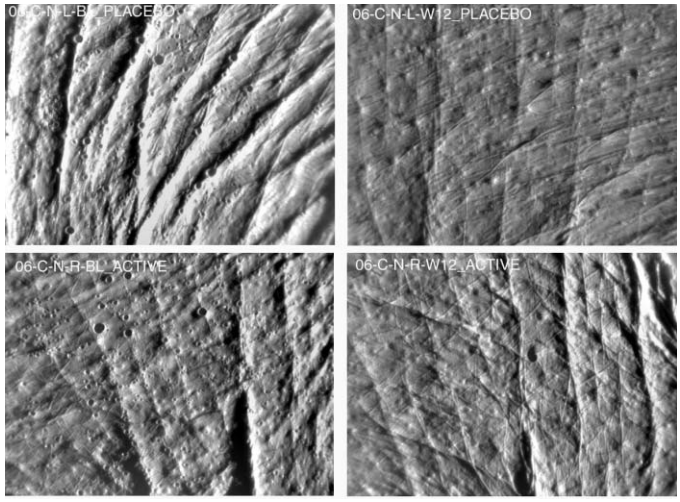


FIGURE 5. Photographs of fine lines on placebo side (top) vs active side (bottom) at baseline (left) and at 12 weeks (right).



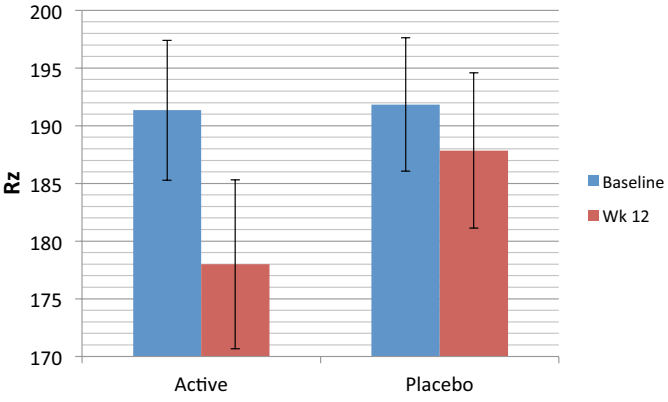
Silastic replicas showed improvement in periocular rhytides on the SCA-treated side (Figure 5) when the light was cast in the normal direction. Crow's feet changes from baseline were consistent and significant ($P \leq .05$) for the total number of coarse lines for the SCA-treated side at 12 weeks vs at baseline. Two other parameters were directionally significant ($P \leq .1$), including the average maximum difference in luminance value for 5 equal-length segments in each of the 10 lines traversing the sample and the length of each coarse line. No significant or directionally significant changes were observed in the placebo-treated side at 12 weeks vs at baseline, although there was improvement (Figure 5). These significant changes were in the direction of smoother and fewer wrinkles (Figure 6). Differences between the active and placebo sides were not great enough to be confirmed in the direct comparison test. Light cast in the parallel direction demonstrated slight improvement in fine lines in both the SCA-treated and placebo-treated skin.

DISCUSSION

A screen for natural products bearing regenerative properties yielded a secretion of the SCA, which was found to possess skin-regenerative properties.[7] It was noted that when snails perceive radiation, they retract their orientation organs, and as a defensive mechanism, they secrete large amounts of mucous substances to protect themselves from harmful radiation. This prompted Ledo et al to create an experimental rat model, where an acute radiodermatitis was induced and secretions from the mollusk were applied that showed skin regeneration.[8]

To elucidate the mechanism of action behind the regenerative properties of SCA, Brieva et al performed several in vitro analyses to explain the physiological effect of SCA.[9] It was found that SCA possessed antioxidant properties by not only being able to scavenge free radicals, as demonstrated when assayed, but

FIGURE 6. The maximum difference in luminance value (Rz) of crow's feet wrinkles illuminated from the normal (perpendicular) direction after 12 weeks (n=23).



also by demonstrating superoxide dismutase (SOD) activities. SOD is a key enzyme involved in inactivating the superoxide anion (O_2^-) radical and hydrogen peroxide.[10] SCA also increased fibroblast cell proliferation and promoted increase cell survival upon irradiation with UV-A light. A complementary mechanism is provided by the fact that SCA promotes extracellular matrix assembly, as demonstrated by its ability to induce fibronectin assembly, which is essential for wound healing and tissue plasticity.[11-14] Finally, SCA was shown to downregulate matrix metalloproteinase expression in dermal fibroblasts, which limits the extent of the damage during wound healing and scar formation.[14] Together, it is believed that these mechanisms contribute to the observed beneficial effects of SCA and, the authors postulate, its employment in regenerative therapy.

Results of an initial, pilot, nonrandomized, open-label study using SCA for the treatment of cutaneous photoaging in 15 women were promising.[15] Sallowness decreased by 50% within the first 30 days of SCA use, and by more than 75% by day 90. A significant ($P<.05$) reduction was noted in fine lines, deep wrinkles, as well as elasticity. Dryness and roughness resolved in all patients by day 90 as well. Improvement in collagen was reflected in a softening of coarse lines and wrinkles as well as a global improvement/reduction of wrinkles and improvement in skin texture. Outcomes suggested an increase in hyaluronic acid content. Improved skin tone and elasticity suggested beneficial effects of SCA on elastin fibers. Biopsy results showed a reduction of solar elastosis through improved elastin quality following 90 days of SCA application, a result that is significant in itself. Investigators concluded that twice-daily application of SCA would yield continued improvement in all parameters assessed during the study, and that these results served as adequate preliminary evidence supporting the benefits of regular use.

Results from our placebo-controlled, split-face study corroborate those of the open-label Tribó-Boixareu report, where a significant improvement in periocular rhytides was noted after 12 weeks of use of SCA serum and emulsion.[15] Also, a greater improvement in skin texture was seen on the SCA-treated side at all visits when compared with placebo.

CONCLUSION

Daily application of 8% SCA emulsion and 40% SCA serum significantly improved periocular rhytides after 12 weeks, when compared with inactive placebo. Furthermore, both periocular and perioral texture improved to a greater degree in the active side when compared with the placebo side at 8, 12, and 14 weeks (2 weeks after being off the product). In addition to being efficacious, SCA proved to be well tolerated, with no adverse reactions reported throughout the study time frame.

DISCLOSURES

This study was supported by a research grant from Biopelle. Dr. Cohen has served as a consultant and clinical trial participant for Biopelle (a division of Ferndale Pharmaceutical Company. Ferndale, MI). Dr. Fabi has served as a speaker for Biopelle.

REFERENCES

1. Babu M, Wells A. Dermal-epidermal communication in wound healing. *Wounds*. 2001;13(5):183-189.
2. Bonin-Debs AL, Boche I, Gille H, Brinkmann U. Development of secreted proteins as biotherapeutic agents. *Expert Opin Biol Ther*. 2004;4(4):551-558.
3. Liu B, Earl HM, Baban D, et al. Melanoma cell lines express VEGF receptor KDR and respond to exogenously added VEGF. *Biochem Biophys Res Commun*. 1995;217(3):721-727.
4. Draelos ZD. Exploring the pitfalls in clinical cosmeceutical research. *Cosmet Dermatol*. 2007;20(9):556-558.
5. Lázár-Molnár E, Hegyesi H, Tóth S, Falus A. Autocrine and paracrine regulation by cytokines and growth factors in melanoma. *Cytokine*. 2000;12(6):547-554.
6. Abad RF. Treatment of experimental radiodermatitis with a regenerative glucoproteic mucopolysaccharide complex. *Dermatol Cosmet*. 1999;9:53-57.
7. Brieva A, Philips N, Tejedor R, et al. Molecular basis for the regenerative properties of a secretion of the mollusk Cryptomphalus aspersa. *Skin Pharmacol Physiol*. 2008;21(1):15-22.
8. Ledo E, de las Heras ME, Ledo A. Treatment for acute radiodermatitis with Cryptomphalus aspersa secretion. *Radioprotección*. 1999;23:34-38.
9. Brieva A, Guerrero A, Pivel JP. Mecanismos bioquimicos y farmacologicos relacionados con la actividad de la secrecion de Cryptomphalus aspersa (SCA) (Endocare®) en radiodermitis. *Dermatol Cosmet*. 2001;11:71-75.
10. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation. 2003;108(16):1912-1916.
11. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol. 2007;127(3):514-525.
12. Schwartz E, Cruickshank FA, Christensen CC, Perlish JS, Lebwohl M. Collagen alterations in chronically sun-damaged human skin. *Photochem Photobiol*. 1993;58(6):841-844.
13. Bertaux B, Horneback W, Eisen AZ, Dubertret L. Growth stimulation of human keratinocytes by tissue inhibitor of metalloproteinases. *J Invest Dermatol*. 1991;97(4):679-685.
14. Cruz MC, Sanz-Rodríguez F, Zamarrón A, et al. A secretion of the mollusc Cryptomphalus aspersa promotes proliferation, migration and survival of keratinocytes and dermal fibroblasts in vitro. *Int J Cosmet Sci*. 2012;34(2):183-189.
15. Tribó-Boixareu MJ, Parrado-Romero C, Rais B, Reyes E, Vitale-Villarejo MA, González S. Clinical and histological efficacy of a secretion of the mollusk Cryptomphalus aspersa in the treatment of cutaneous photoaging. *Cosmet Dermatol*. 2009;22(5):247-252.

AUTHOR CORRESPONDENCE

Sabrina Fabi MD
E-mail:.....sfabi@gbkderm.com