An Open Label Clinical Trial of a Multi-Ingredient Anti-Aging Moisturizer Designed to Improve the Appearance of Facial Skin

James H. Herndon Jr. MD,[a] Lily Jiang PhD,[a] Tatiana Kononov BS MBA,[b] Theresa Fox BS[b]

[a]Thomas J. Stephens and Associates, Richardson, TX
[b]Revision Skincare, Irving, TX

ABSTRACT

An open label clinical trial was conducted to determine the effectiveness of a multi-ingredient anti-aging moisturizer designed to improve the appearance of facial skin. Parameters studied included fine lines and wrinkles, clarity/brightness, visual roughness, tactile roughness, evenness of skin tone (redness), evenness of skin tone (hyperpigmentation) and overall appearance. Thirty-seven female subjects, ages 35-60 years completed the study. Effective ingredients incorporated into the facial anti-aging moisturizer include: *Astragalus membranaceus* root extract, a peptide blend including palmitoyl tripeptide-38, standardized rosemary leaf extract (ursolic acid), tetrahexyldecyl ascorbate (THD ascorbate) and ubiquinone (coenzyme Q10). Subjects were instructed to apply the moisturizer twice daily, once in the morning and once in the evening. Subjects were evaluated at baseline and after 4, 8, and 12 weeks of product usage. Clinical evaluations were conducted at each visit. A self-assessment questionnaire was conducted at week 4, week 8, and week 12. The self-assessment questionnaire included product efficacy inquiries and product aesthetic inquiries. Digital photography was conducted at baseline, week 8, and week 12. After 8 weeks of twice daily use, clinical evaluation results show that the multi-ingredient anti-aging moisturizer produced a statistically significant improvement in the scores of all clinical grading parameters assessed compared to baseline. A greater statistically significant improvement was seen at 12 weeks. At week 12, there was a statistically significant percentage of favorable results versus unfavorable results in all product efficacy and product aesthetic self-assessment questionnaire results. Digital photography supported the clinical grading and self-assessment questionnaire results. Additionally, the multi-ingredient anti-aging moisturizer is judged to be mild and well tolerated. Several tolerability parameters were assessed at all time points with no statistically significant increase in any of the scores compared to baseline.

J Drugs Dermatol. 2015;14(7):699-704.

INTRODUCTION

The condition of the skin on the face deteriorates with age.[1] Signs of aging facial skin include: fine lines and wrinkles, reduction in clarity/brightness of the skin, increase in visual and tactile roughness, increase in redness, and increase in hyperpigmentation. A multi-ingredient anti-aging moisturizer was designed to treat the skin on the face in order to improve the signs of aging. Effective ingredients incorporated into the treatment include: *Astragalus membranaceus* root extract, a peptide blend including palmitoyl tripeptide-38, standardized rosemary leaf extract (ursolic acid), tetrahexyldecyl ascorbate (THD ascorbate) and ubiquinone (coenzyme Q10). The combination of the effective ingredients listed above is expected to have a beneficial impact on the signs of aging skin. Previous studies have demonstrated the effectiveness of these ingredients. Cycloartane-type saponins, phytocompounds found in the Astragalus root, have been shown to promote wound healing through increased fibroblast proliferation and migration.[2] Hong et al found that *Astragalus embranaceus* root had an inhibitory effect on matrix metalloproteinase-1 collagenase expression and procollagen expression on UVB irradiated human dermal fibroblasts.[3] Concerning peptides used in skincare, Lintner and Peschard demonstrated that topically delivered peptides with a fatty acid group attachment have

biological activity in improving the skin and its properties.[4] The specific peptide, palmitoyl tripeptide-38, has shown activity in upregulating collagens I and III, fibronectin, and hylauronic acid in the dermis while also boosting key proteins of the dermal-epidermal junction, collagen IV and laminins.[5] Soo Lee's work demonstrates that ursolic acid may be useful in inhibiting UVA-induced photoaging.[6] It is known that Vitamin C is very beneficial for treating aging skin.[7,8] THD ascorbate has been shown to be an efficacious and stable form of Vitamin C.[9] Ochiai et al showed that THD ascorbate has skin lightening effects.[10] Xiao et al demonstrated that THD Ascorbate has protective effects in the presence of UVA radiation.[11] Coenzyme Q10 has multiple known topical benefits including antioxidant, energizing and wrinkle inhibitory effects.[12,13] Incorporating this described combination of ingredients at efficacious levels into a moisturizing cream base should produce a statistically significant anti-aging result.

OBJECTIVE

To assess the long-term efficacy of a multi-ingredient anti-aging moisturizer in improving the following parameters: 1) fine lines 2) wrinkles 3) clarity/brightness 4) visual roughness 5) tactile roughness 6) evenness of skin tone (redness) 7) even-

J.H. Herndon Jr., L. Jiang, T. Kononov, T. Fox

ness of skin tone (hyperpigmentation) 8) overall appearance. Additionally, to assess the perception of the product efficacy and product aesthetic attributes by users through a self-assessment questionnaire.
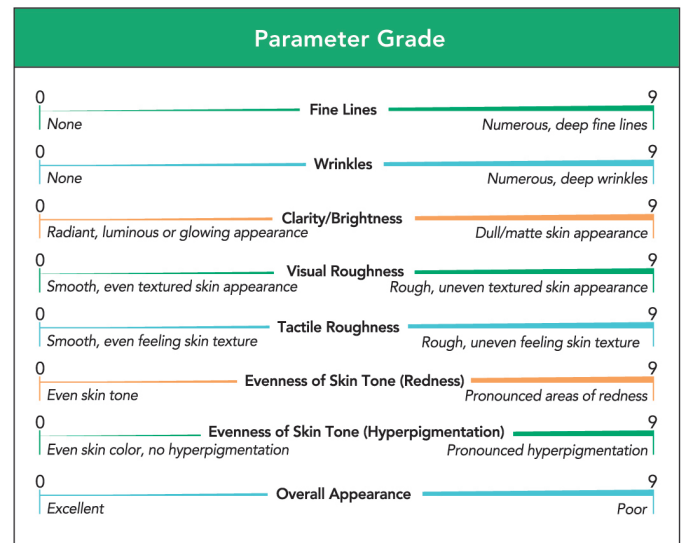
Material and Methods

A multi-ingredient anti-aging moisturizer was formulated to address the multiple signs of aging of the facial skin. This formulation included *Astragalus membranaceus* root extract, a peptide blend including palmitoyl tripeptide-38, standardized rosemary leaf extract (ursolic acid), tetrahexyldecyl ascorbate (THD ascorbate) and ubiquinone (coenzyme Q10), in a moisturizing oil-in-water emulsion base. A 12-week efficacy study was completed by 37 female subjects in the age range of 35–60 years. Qualified subjects exhibited mild to moderate facial wrinkles and uneven skin tone. The demographic information is shown in Figure 1. The subjects were instructed to apply the treatment evenly to the face twice daily, once in the morning and once in the evening. Subject visits to the testing facility occurred at baseline, week 4, week 8 and week 12. Subject diaries were collected at each visit and reviewed for compliance and comments. Additionally, units of the test materials were collected, each visually inspected, and weighed for study compliance. Clinical evaluations of each subject were conducted during all visits by an expert grader using the scale shown in Figure 2. A self-assessment questionnaire was also conducted at week 4, week 8, and week 12, which included both product efficacy and product aesthetic inquiries.

FIGURE 1. Demographic information for subjects completing 12-week study.

Demographic Information		
All subjects (n=37)		
	n	**%**
Skin Type		
Dry	1	2.7
Normal-To-Dry	21	56.8
Normal	15	40.5
Age (Years)		
Mean	52.1	
Standard Deviation	6.4	
Minimum	35	
Median	54.0	
Maximum	60	

FIGURE 2. Parameter grading scale used by expert grader during clinical grading efficacy.

For clinical grading efficacy, mean percentage change and percentage of subjects improved or worsened were calculated and reported at the post-baseline time points. Treatment comparisons were made in terms of change from baseline at all post-baseline time points. The null hypothesis that the mean change from baseline is zero was tested using the Wilcoxon signed-rank test. All statistical tests were performed at significance level alpha=0.05. Statistical analyses were performed using SAS software version 9.30 series (SAS Statistical Institute). Electronic data capture (EDC) methods were used to record clinical grading efficacy. The EDC used is compliant with FDA regulations.

For self-assessment questionnaires, subjects completed a sponsor-provided self-assessment questionnaire that included product efficacy inquiries regarding subjects' perceptions of skin condition parameters as well as product aesthetic inquiries regarding subjects' perceptions of the product attribute parameters. Inquiries were completed at each post-baseline time point and were tabulated and the frequency and percentage of all response options was reported for each question and time point. A binomial (sign) test was performed to test if the proportion of the combined designated favorable responses was equal to the combined designated negative responses for applicable questions.

Digital photography was used to document each subject's condition during the baseline, week 8, and week 12 visit. Each subject had a total of 3 full-face images taken under visible light and cross-polarized light using the VISIA CR photo-station (Canfield Imaging Systems, Fairfield, NJ) with a Canon Mark II 5D digital SLR camera (Canon Incorporated, Tokyo, Japan). The 3 full-face images consisted of a right side view, left side view,

J.H. Herndon Jr., L. Jiang, T. Kononov, T. Fox

and center view. Digital photographs taken under cross-polarized lighting at baseline and week 12 were subjected to image analysis to assess skin tone evenness. The analysis was done using proprietary macros developed by Stephens & Associates using Image Pro Plus v7 software (Media Cybernetics, Inc., Rockville, Maryland). Skin tone evenness analysis quantifies the variation of color intensity on defined areas of the face and reports skin tone evenness index, which is defined as the average of (standard deviation)^2/(5*average intensity) of each color channel. Lower index value indicates more even skin tone. The cheek area from left view photos were selected for analysis.

> "Incorporating this described combination of ingredients at efficacious levels into a moisturizing cream base should produce a statistically significant anti–aging result."

RESULTS

A statistically significant improvement was detected by the expert grader for all clinical grading criteria assessed at weeks 8 and 12 for the multi-ingredient anti-aging moisturizer when compared to baseline scores (Figures 3-6). This criteria includes: fine lines, wrinkles, clarity/brightness, visual roughness, tactile roughness, evenness of skin tone (redness), evenness of skin tone (hyperpigmentation) and overall appearance. After 12 weeks of twice daily use:

• 100% of subjects showed an improvement in fine lines with an average improvement of 25.1%.
• 97% of subjects showed an improvement in wrinkles with an average improvement of 16.9%.
• 97% of subjects showed an improvement in clarity/brightness with an average improvement of 22.6%.
• 100% of subjects showed an improvement in visual roughness with an average improvement of 26.4%.
• 97% of subjects showed an improvement in tactile roughness with an improvement of 32.5%.
• 89% of subjects showed an improvement in evenness of skin tone (redness) with an average improvement of 79.1%.
• 100% of subjects showed an improvement in evenness of skin tone (hyperpigmentation) with an average improvement of 19.0%.
• 100% of subjects showed an improvement in overall appearance with an average improvement of 19.8%.

These improvements were captured through digital photographs taken before and after 12 weeks of product usage. As

shown in Figures 7 and 8, features such as reduction in fine lines and wrinkles, improvement in skin tone evenness, improvement in visual smoothness, improvement in clarity/brightness, and improvement in overall appearance, are visible. In addition, objective and quantitative assessment of skin tone evenness using image analysis indicated that 78% of subjects showed an improvement in skin tone evenness with an average improvement of 8.6% after 12 weeks of product use. This change is statistically significant and consistent with clinical grading results (Figure 9).

FIGURE 3. Percent of subjects that showed an improvement in wrinkles at the week 4, week 8, and week 12 time points compared to baseline during a 12-week study using a facial anti-aging moisturizing treatment twice daily. *P*<.001.
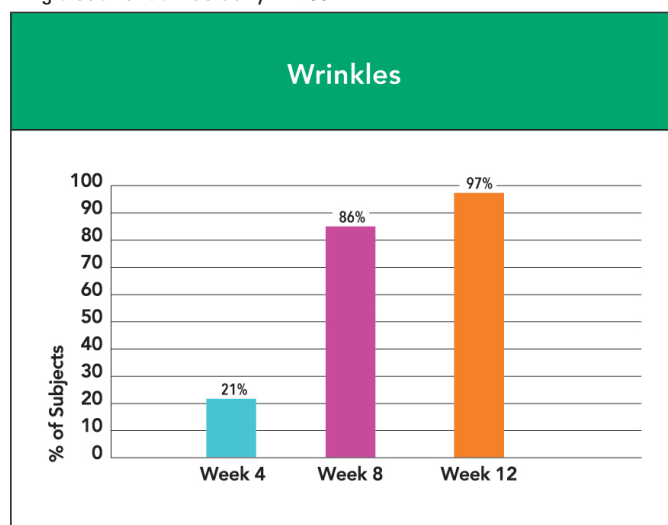

FIGURE 4. Percent of subjects that showed an improvement in evenness of skin tone (hyperpigmentation) at the week 4, week 8, and week 12 time points compared to baseline during a 12-week study using a facial anti-aging moisturizing treatment twice daily. *P*<.001.

JO0715

702

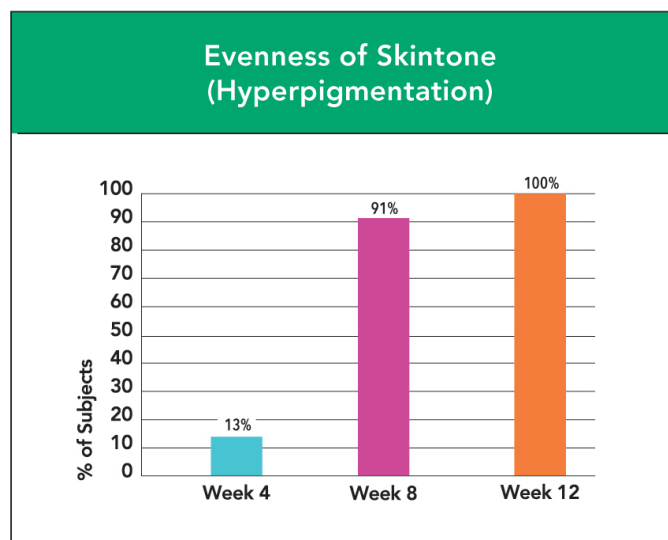J.H. Herndon Jr., L. Jiang, T. Kononov, T. Fox

FIGURE 5. Percent of subjects that showed an improvement in various parameters at the week 12 time point compared to baseline during a 12-week study using a facial anti-aging moisturizing treatment twice daily. *P*<.001.

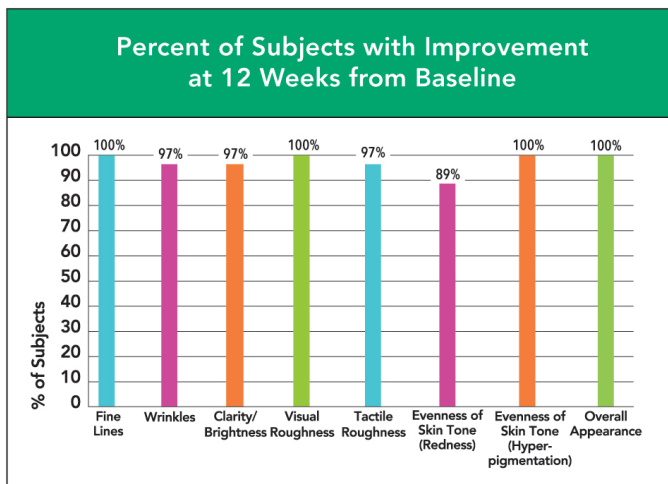Percent of Subjects with Improvement at 12 Weeks from Baseline

FIGURE 6. Average improvement of subjects in various parameters at the week 12 time point compared to baseline during a 12-week study using a facial anti-aging moisturizing treatment twice daily. *P*<.001.
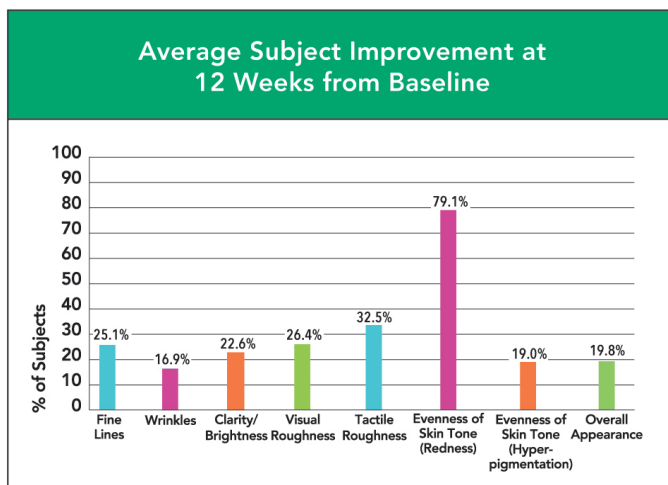

Average Subject Improvement at 12 Weeks from Baseline

A statistically significant percentage of subjects responded favorably to all product efficacy inquiries included in the self-assessment questionnaire. After 12 weeks of twice daily use:
• 81% of subjects thought that their skin texture looked improved.
• 75% of subjects thought that their face looked more radiant or brighter.
• 73% of subjects thought that the fine lines on their face were less noticeable.
• 78% of subjects thought that their skin looked and felt healthier.
• 75% of subjects thought that their skin looked younger and firmer.
• 78% of subjects thought that their skin felt more moisturized.
• 78% of subjects thought that overall, their skin appearance improved.

A statistically significant percentage of subjects responded favorably to all product aesthetic inquiries included in the self-assessment questionnaire. After 12 weeks of twice daily use:
• 83% of subjects thought that the product moisturized their skin.
• 86% of subjects thought that the product scent was pleasant.
• 86% of subjects thought that the product texture was pleasant.
• 94% of subjects thought that the product did not leave an unpleasant after-feel.
• 86% of subjects thought that applying the product was a pleasant experience.
• 94% of subjects thought that overall, the product did not irritate their skin.

Safety/Tolerability: The multi-ingredient anti-aging moisturizer was well tolerated by the panelists with no statistically significant increase in scores for tolerability parameters at all time points when compared to baseline scores.

DISCUSSION

The signs of aging of the facial skin such as fine lines, wrinkles, diminished clarity/brightness, increase in visual and tactile roughness, reduction of evenness of skin tone (increase in redness) and reduction in evenness of skin tone (increase in hyperpigmentation) are a complex issue with multiple causes. These causes are related to exposure to ultra-violet radiation, uneven synthesis and distribution of melanin, a deterioration of the dermal-epidermal junction (DEJ), free-radical damage including the degradation of collagen and elastin in the skin as well as the compromised synthesis of new collagen and elastin proteins.

The multi-ingredient anti-aging moisturizer contains various ingredients thought to address these varying causes:
 • Astragalus Membranaceus Root Extract – contains antioxidants and cycloartane-type saponins, helps overall well-being of the skin
• Peptide Blend including Palmitoyl Tripeptide-38 – helps increase collagens I and III as well as the production of DEJ proteins
 Standardized Rosemary Leaf Extract (ursolic acid) – a proven, potent antioxidant with moisture barrier protection properties, protective effect in the presence of UVA radiation
• THD Ascorbate – Vitamin C derivative, antioxidant with skin brightening and multiple anti-aging properties
• Ubiquinone (Coenzyme Q10) – antioxidant, reduces free radical formation

It is hypothesized that a combination of effective ingredients such as those listed above formulated into a moisturizing oil-in-water emulsion base will result in an improvement in the signs of aging around the facial skin. The concerned study appears to positively support the hypothesis as is seen by the results illustrated in Figures 3-9.

JO0715

703

J.H. Herndon Jr., L. Jiang, T. Kononov, T. Fox

FIGURE 7. Treatment of aging skin with facial anti-aging moisturizing treatment at baseline and after 12 weeks of twice daily use.

Before | After 12 weeks

FIGURE 8. Treatment of aging skin with facial anti-aging moisturizing treatment at baseline and after 12 weeks of twice daily use.

Before | After 12 weeks

FIGURE 9. Quantitative and objective assessment of skin tone evenness using image analysis at 12-week time perioid.

12-Week Image Analysis of Skin Tone

	%
Subjects Showing an Improvement	78
Average Improvement	8.6

> "A statistically significant improvement was detected by the expert grader for all clinical grading criteria assessed at weeks 8 and 12 for the multi–ingredient anti–aging moisturizer when compared to baseline scores."

CONCLUSION

A 12-week clinical usage study was conducted to assess the long-term efficacy of a multi-ingredient anti-aging moisturizer in reducing the signs of aging of the facial skin. Limitations of the study include no vehicle control. The multi-ingredient anti-aging moisturizer was shown to be effective in reducing the signs of aging of the facial skin including fine lines and wrinkles, clarity/brightness, visual roughness, tactile roughness, evenness of skin tone (redness), evenness of skin tone (hyperpigmentation) and overall appearance after 8 and 12 weeks of twice daily use when compared to baseline scores. The subjects also liked the product and believed in the efficacy of the product as noted by the self-assessment questionnaire results. The statistically significant results provide medical professionals with information that could be beneficial when consulting a patient with signs of aging of the facial skin.

ACKNOWLEDGMENT

DISCLOSURES

Dr. Herndon and Dr. Jiang have no conflicts of interest to declare. Tatiana Kononov and Theresa Fox are employees of Revision Skincare

REFERENCES

1. Elicidus Kurban RS, Bhawan J. Histologic changes in skin associated with aging. *J Dermatol Surg Oncol.* 1990;16(10):908-14.
2. Sevimli-gür C, Onbaşılar I, Atilla P, et al. In vitro growth stimulatory and in vivo wound healing studies on cycloartane-type saponins of *Astragalus* genus. *J Ethnopharmacol.* 2011;134(3):844-50.
3. Hong MJ, Ko EB, Park SK, Chang MS. Inhibitory effect of *Astragalus membranaceus* root on matrix metalloproteinase-1 collagenase expression and procollagen destruction in ultraviolet B-irradiated human dermal fibroblasts by suppressing nuclear factor kappa-B activity. *J Pharm Pharmacol.* 2013;65(1):142-8.
4. Lintner K, Peschard O. Biologically active peptides: from a laboratory bench curiosity to a functional skin care product. *Int J Cosmet Sci.* 2000;22(3):207-18.
5. Fournial A, Mondon P, Peschard O, inventors; Sederma, assignee. New compounds, in particular, peptides, compositions comprising them and cosmetic and dermo-pharmaceutical use. US patent 8697656 B2. April 15, 2014.
6. Soo Lee Y, Jin DQ, Beak SM, Lee ES, Kim JA. Inhibition of ultraviolet-A-modulated signaling pathways by asiatic acid and ursolic acid in HaCaT human keratinocytes. *Eur J Pharmacol.* 2003;476(3):173-8.
7. Farris PK. Topical vitamin C: a useful agent for treating photoaging and other dermatologic conditions. *Dermatol Surg.* 2005;31(7 Pt 2):814-7.
8. Sauermann K, Jaspers S, Koop U, Wenck H. Topically applied vitamin C increases the density of dermal papillae in aged human skin. *BMC Dermatol.* 2004;4(1):13.

JO0715

J.H. Herndon Jr., L. Jiang, T. Kononov, T. Fox

9.   Maia Campos PM, Gianeti MD, Camargo FB, Gaspar LR. Application of tetra-isopalmitoyl ascorbic acid in cosmetic formulations: stability studies and in vivo efficacy. *Eur J Pharm Biopharm*. 2012;82(3):580-6.

10. Ochiai Y, Kaburagi S, Obayashi K, et al. A new lipophilic pro-vitamin C, tetra-isopalmitoyl ascorbic acid (VC-IP), prevents UV-induced skin pigmentation through its anti-oxidative properties. *J Dermatol Sci*. 2006;44(1):37-44.

11. Xiao L, Kaneyasu K, Saitoh Y, Terashima Y, Kowata Y, Miwa N. Cytoprotective effects of the lipoidic-liquiform pro-vitamin C tetra-isopalmitoyl-ascorbate (VC-IP) against ultraviolet-A ray-induced injuries in human skin cells together with collagen retention, MMP inhibition and p53 gene repression. *J Cell Biochem*. 2009;106(4):589-98.

12. Hoppe U, Bergemann J, Diembeck W, et al. Coenzyme Q10, a cutaneous antioxidant and energizer. *Biofactors*. 1999;9(2-4):371-8.

13. Inui M, Ooe M, Fujii K, Matsunaka H, Yoshida M, Ichihashi M. Mechanisms of inhibitory effects of CoQ10 on UVB-induced wrinkle formation in vitro and in vivo. *Biofactors*. 2008;32(1-4):237-43. tem

AUTHOR CORRESPONDENCE

Tatiana Kononov BS MBA

E-mail:................…….................... tkononov@revisionskincare.com