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Red Ginseng Root Extract Mixed with Torilus Fructus and Corni Fructus Improves Facial Wrinkles and Increases Type I Procollagen Synthesis in Human Skin: A Randomized, Double-Blind, Placebo-Controlled Study

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ABSTRACT Red ginseng contains many bioactive constituents, including various ginsenosides that are believed to have antioxidant, immunostimulatory, and anti-aging activities. Yet, no controlled human study has explored its effects on photoaged skin. This study determined whether long-term intake of a red ginseng extract-containing Torilus fructus and Corni fructus mixture reduces facial wrinkles and increases collagen synthesis in human skin. Healthy female volunteers over 40 years of age were randomized in a double-blind fashion to receive either red ginseng extract-containing herbal mixture at 3 g/day or placebo for 24 weeks. Facial wrinkles, elasticity, epidermal water content, erythema, and pigmentation were measured objectively. Facial skin samples were taken before and after treatment, and real-time polymerase chain reaction and immunohistochemical analyses were undertaken for expression of type I procollagen, matrix metalloproteinase (MMP)-9, and fibrillin-1, which are wrinkle-related biochemical markers. A total of 82 subjects completed the study. Facial wrinkles were significantly improved, type I procollagen gene and protein expression was increased, MMP-9 gene induction was prevented, and fibrillin-1 fiber length was elongated only in the treatment group. No changes were seen in the facial elasticity, epidermal water content, facial erythema and pigmentation, and epidermal thickness in either group. Thus a red ginseng extract-containing Torilus fructus and Corni fructus mixture improves facial wrinkles, a clinical sign of photoaging, and this improvement is associated with biochemical and histological evidence of increased collagen synthesis in the dermis. These results substantiate the alleged beneficial effects of red ginseng on photoaging and support its use as an effective "beauty food."

KEY WORDS: • *aging* • *dietary supplement* • *matrix metalloproteinase* • *red ginseng*

INTRODUCTION

SOLAR ULTRAVIOLET (UV) radiation causes sunburn, hyperpigmentation, immunosuppression, photocarcinogenesis, and premature skin aging (photoaging).[1,2] Photoaging is a form of premature skin aging that is superimposed on chronological aging in chronically photodamaged skin and is characterized by severe wrinkling, roughness, laxity, and pigmentary changes, such as solar lentigo and a mottled pigmentation on sun-exposed areas.[3] In addition, photoaging is accompanied by histological changes, such as prominent cellular component alterations and damage to the extracellular connective tissue matrix—for example, collagen fiber damage, the excessive deposition of abnormal elastic fibers (*i.e.*, elastosis), and increases in glycosaminoglycan levels.[4,5] The exposure of human or mouse skin to UV results in the induction of a series of matrix metalloproteinases (MMPs), which have been implicated in the photoaging process.[6]

The MMPs are a family of structurally related molecules including collagenase-1 (MMP-1), collagenase-3 (MMP-13), gelatinase A and B (MMP-2 and MMP-9), the stromelysins (MMP-3), membrane-type MMPs, and others, which are capable of degrading all components of the extracellular matrix such as collagen, elastin, fibronectin, proteoglycans, and laminin.[7]

In particular, MMP-1 (interstitial collagenase or collagenase 1) initiates the degradation of type I and III fibrillar

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collagen, MMP-9 (gelatinase B) further degrades the collagen fragments produced, and MMP-3 (stromelysin 1) degrades type IV collagen and activates pro-MMP-1.[8–10] Chronic UV exposure induces the expression of MMPs (collagenases, gelatinase B, and stromelysin), which can degrade skin connective tissue and thus contribute to premature skin aging (photoaging).[8–10] Moreover, interstitial collagenase (MMP-13 in mice) and gelatinase B (MMP-3) degrade collagen, which leads to collagen deficiency and wrinkling.[2] Resultant decreases in collagen and elastic fiber of the wrinkled skin can be assessed by determining procollagen I and fibrillin-1 expression, respectively, along with MMP expression.

Various efforts have been made to understand the relationship between foods and health. Moreover, studies on health-related nutritional foods and oriental herb extracts are providing new insights into the effects of food additives on skin condition and on the prevention of skin aging. The roots of *Panax ginseng* have been traditionally used as a general tonic in Oriental medicine for several thousand years. White ginseng is the peeled and air-dried root of *P. ginseng*, and red ginseng (Ginseng Radix Rubra) is steamed and then air-dried.[11] It has been reported that red ginseng has more bioactivity than white ginseng.[11]

Ginsenosides are the major active constituents of ginseng and are widely held to be responsible for its pharmacological properties.[12] The ginsenosides may be divided into three groups on the basis of chemical structure: protopanaxadiol, protopanaxatriol, and oleanolic saponins.[13] The ginsenosides, which are also referred to as ginseng saponins, have been investigated with regards to many biological and pharmacological effects, such as anti-aging, anti-inflammation, and antioxidation in the central nervous, cardiovascular, and immune systems.[12,13]

During recent years, ginseng saponin metabolites formed by intestinal bacteria were identified after orally administering ginseng extract to humans and rats.[14,15] One of the major metabolites, Compound K [20-*O*-β-D-glucopyranosyl-20(*s*)-protopanaxadiol], has been reported to have antimetastatic, anti-angiogenic, and anti-allergic activities *in vivo*.[16,17] In addition, the topical application of Compound K has been reported to prevent or ameliorate xerosis and skin wrinkling[18] by inducing the *hyaluronan synthase 2* gene in transformed human keratinocytes and increasing the hyaluronan content in aged hairless mouse skin. It has also been reported that ginseng saponin metabolites suppress MMP-9 induction by phorbol 12-myristate 13-acetate.[19] Moreover, a recent *in vitro* study reported that human type I procollagen synthesis is induced, in association with phosphorylation of Smad2, and tumor necrosis factor-α-induced MMP-1 secretion is inhibited in human dermal fibroblasts by *P. ginseng*.[20]

Despite these various reported effects of ginseng, no well-controlled experimental study exists regarding the effects of orally administered red ginseng on cutaneous photoaging *in vivo*. Thus, in this randomized, double-blind, placebo-controlled study, we investigated whether the repeated oral administration of red ginseng extracts and herbal mixtures (KTNG0345 [a proprietary mixture of the Korea Tobacco and Ginseng Corp., Daejeon, Republic of Korea]) can reduce UVB-induced wrinkle formation in human volunteers.

MATERIALS AND METHODS

Subjects

Healthy female subjects over the age of 40 years were randomized to receive KTNG0345 or placebo in a double-blind fashion. When a *t* test was used to calculate the required number of subjects for a meaningful comparison between two groups, 68 subjects were deemed necessary; however, considering this was a 6-month study, the dropout rate was set at 20%, and thus it was determined that at least 82 subjects should be enrolled. Exclusion criteria included age under 40 years, pregnancy or lactation, infectious skin disorder on the face, atopic dermatitis, photoallergic or photosensitive skin, food allergies, intake of any medication, immunosuppressant intake within 3 months prior to enrollment, systemic steroid intake or phototherapy within 1 month prior to enrollment, history of serious renal or hepatic dysfunction, and chronic disease such as asthma, diabetes, and hypertension. Every subject was assigned to either the treatment or placebo group by computer-generated randomization code. In order to compare ingestion frequency of different foods between the treatment and placebo groups, each subject was asked to fill out a Food Frequency Questionnaire on 25 specific foods from eight basic food groups at baseline, 12 weeks, and 24 weeks of the clinical trial. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital. Prior to enrollment, each subject read and signed an informed consent, and the study was implemented in accordance with the Declaration of Helsinki.

Preparation and oral administration of KTNG0345

KTNG0345 used in this study was prepared from Korean red ginseng extracts by the Korea Tobacco and Ginseng Corp. The compound in each capsule consisted of 45.3% (by weight) of Korean red ginseng extract powder and 54.6% of the powdered extracts of two herbs, Torilus fructus and Corni fructus. In brief, the ginseng powder was extracted with 55% ethanol (6 volumes of 55% ethanol, 65–68°C, 24 hours, six times) from Ginseng Radix Rubra, and the herb extracts mix powder was prepared with spray-drying of distilled water extracts from Torilus fructus and Corni fructus (Torilus fructus:Corni fructus = 2:1, 6 volumes of distilled water, 85°C, 4 hours). The levels of the main constituents of the three substances in KTNG0345 (ginsenoside-Rb1, torilin, and loganin) were 10.85 mg/g, 0.12 mg/g, and 3.33 mg/g, respectively. Each hard capsule contained 300 mg of the powdered ginseng and herb mixture, and the daily dosage was 3 g/day (10 capsules). Placebo material consisted of 226.5 mg of dextrin and 70 mg of caramel color. KTNG0345 or placebo was administered orally to the subjects daily for 24 weeks.

Facial wrinkle and elasticity measurements

At baseline, 12 weeks, and 24 weeks, facial wrinkles were measured using skin replicas and a skin visiometer. Skin

replicas (impressions) were prepared by applying silicon rubber (Silflo dental impression materials, Flexico Developments, Ltd., Potters Bar, UK) to the crow's feet area just lateral to the right eye. Skin impressions were photographed using a stereomicroscope (model SZH, Olympus, Osaka, Japan) fitted with an automatic photomicrography system and analyzed using Skin Visiometer SV600 software (Courage and Khazaka Electronic GmbH, Köln, Germany). The Visiometer is a computerized instrument that makes skin microrelief scans from replicas using a light transmission method. It has five roughness parameters: depth of roughness (*R1*), mean depth of roughness (*R2*), maximum roughness (*R3*), depth of smoothness (*R4*), and arithmetic average roughness (*R5*). The Visometer *R* values *R1–R5* decrease as wrinkles diminish.

Facial elasticity was measured with a Cutometer® (model MPA 580, Courage and Khazaka Electronics). The Cutometer takes measurements based on the principle of suction elongation, using an optical measuring unit. It has three important parameters of elasticity. The closer each value of *CR2* (gross elasticity), *CR5* (net elasticity), and *CR7* (elasticity/complete curve) is to 1, the more elastic the skin.

All measurements were performed in a controlled environment room with constant temperature of 20–25°C, and constant humidity of 45–55% at the Clinical Research Institute, Seoul National University Hospital.

Epidermal water content measurements

Water content of the stratum corneum was measured using a skin capacitance meter (model CM 825 corneometer, Courage & Khazaka Electronic GmbH). This device was used to evaluate the water contents of superficial epidermis of facial skin.

Facial erythema and pigmentation measurements

The degree of facial erythema and pigmentation was measured using a DermaSpectrometer® (Cortex Technology, Hadsund, Denmark). The face cheek was selected for measurement. All measurements were taken the same time the wrinkles, elasticity, and water content of epidermis were assessed.

Clinical assessment of facial wrinkles, elasticity, and pigmentation

Improvements of facial wrinkles, elasticity, and pigmentation were evaluated by the subjects themselves and an investigator at 12 and 24 weeks. Degree of improvement was graded using the following clinical parameters: worse, slightly worse, no change, slightly improved, or improved.

Adverse effects

Complete blood count, aspartate aminotransferase, and alanine aminotransferase were checked at baseline and 24 weeks. We checked for compliance and any adverse effect at 12-week and 24-week follow-up visits.

Real-time polymerase chain reaction (PCR)

At baseline and at week 24, we obtained facial skin samples of eight subjects from each group by performing a 2-mm punch biopsy from the crow's feet area just lateral to the right eye and analyzed the samples biochemically and immunohistochemically. In brief, total RNA was isolated from skin samples using TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). One microgram of total RNA was reverse-transcribed into cDNA using the First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania). Quantitation of procollagen α1(I), MMP-1, and MMP-9 was performed using a fluorescence detection method, the 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Sequence-specific PCR primer sets and *TaqMan* MGB probe (FAM™ dye-labeled) were purchased from Applied Biosystems. For real-time PCR, cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. To quantify the relative change in gene expression between each sample, we used the comparative CT method.

Immunohistochemical analysis

Two-millimeter punch biopsy samples from eight subjects in each group were fixed in 10% formalin for 24 hours and embedded in paraffin. Serial sections (4 μm) were mounted onto silane-coated slides (Dako, Glostrup, Denmark) and allowed to dry at 58°C for 1 hour. Sections were stained with hematoxylin and eosin. Acetone-fixed frozen sections were stained with mouse anti-procollagen type I (SP1.D8) antibody (diluted 1:10, Yale University, New Haven, CT, USA), mouse anti-fibrillin-1 antibody (diluted 1:200, Neomarkers, Freemont, CA, USA), and rabbit anti-tropoelastin antibody (1:1,000, EPC, Owensville, MO, USA) in a humidified chamber at 4°C for 18 hours. After washing in phosphate-buffered saline, sections were visualized using a Histostatin®-Plus kit (Zymed, San Francisco, CA, USA) with a biotinylated secondary antibody and a horseradish-streptavidin conjugate. 3-Amino-9-ethylcarbazole was used as a chromogenic substrate (Dako).

Procollagen type I (SP1.D8) staining was graded semi-quantitatively by five observers who had no information about this study. Using an image analysis program (BMI Plus software, BumMi Universe Co., Kyungki, Republic of Korea), the total percentage area and length of fibrillin were measured from the dermo-epidermal junction to 15 μm downward into the dermis. Total percentage area and length of tropoelastin were measured from the dermo-epidermal junction to 50 μm downward into the dermis. The epidermal thickness (the average distance from the granular layer to basal layer) was measured three times per slide, and the average was taken using the same image analysis program.

Statistical analysis

Student's *t* test, the Mann-Whitney test, and the Wilcoxon signed rank test were used to evaluate the significance of

differences between the effect of KTNG0345 and placebo. Statistical significance was defined as $P < .05$. Statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA). For statistical analysis we consulted the Seoul National University Statistical Research Institute.

RESULTS

Demographics of subjects in each group

A total of 86 female subjects were randomized, and 82 subjects completed the study. Compliance was confirmed by checking each subject's medication intake data sheet and the number of remaining capsules. Three subjects in the treatment group dropped out because of concurrent botulinum toxin treatment on the face during the study, gastrointestinal discomfort, and personal reasons not related to the study, respectively. One subject from the placebo group discontinued because of gastrointestinal discomfort. There was no difference between the two groups in the frequency of intake of different food groups either before or after the clinical trial. There was no difference in the incidence of adverse events between the two groups. The ages of the 82 subjects ranged from 40 to 70 years (average age, 51.9 years), and body weights ranged from 43 to 81 kg (average weight, 57.0 kg). In the treatment group ($n = 40$), average age was 53.6 ± 7.4 years, and average weight was 57.2 ± 7.1 kg; in the placebo group ($n = 42$), average age was 50.3 ± 7.6 years, and average weight was 56.8 ± 7.5 kg. Demographic data of the subjects are summarized in Table 1. Student's t test revealed no significant difference in initial age, weight, Visiometer *R* values, Cutometer *CR* values, and erythema and melanin indices between the two groups, and thus the selection of subjects was deemed appropriate. Laboratory evaluations revealed no significant abnormalities in complete blood count, aspartate aminotranferase, and alanine aminotransferase upon completion of study in either group.

Red ginseng extract improves facial wrinkles in human skin

Comparison of facial wrinkles before and after 12 and 24 weeks of ginseng extract consumption between treatment group and placebo group, as measured by skin replica and Visiometer, is shown in Table 2. Raw data were baseline-adjusted, and then baseline-adjusted ratios were calculated. At 12 weeks, the treatment group demonstrated 14.7% ($P = .004$) and 19.0% ($P = .010$) improvement in *R1* and *R5* values, respectively, compared to the placebo group. At 24 weeks, the magnitude of improvement in the treatment group was 14.1% ($P = .027$) and 23.5% ($P = .007$) in *R1* and *R5*, respectively.

Table 1. Demographics of Subjects in Each Group

	Treatment group	*Placebo group*
Number enrolled	43	43
Number completed	40	42
Average age (years, mean ± SEM)	53.6 ± 7.4	50.3 ± 7.6
Average weight (kg, mean ± SEM)	57.2 ± 7.1	56.8 ± 7.5

Table 2. Percentage Change of Visiometer Values in Treatment and Placebo Groups

	Baseline	*12 weeks*	*24 weeks*
R1			
Ginseng	0	−8.51 ± 3.48*	−5.25 ± 4.62*
Placebo	0	6.22 ± 3.51	8.80 ± 4.20
R2			
Ginseng	0	−7.78 ± 4.02	−3.05 ± 5.48
Placebo	0	1.33 ± 3.86	5.83 ± 4.35
R3			
Ginseng	0	−5.26 ± 3.22	−3.07 ± 4.63
Placebo	0	−0.96 ± 3.05	3.83 ± 3.50
R4			
Ginseng	0	0.75 ± 4.44	−5.79 ± 4.84
Placebo	0	8.30 ± 4.35	7.12 ± 4.86
R5			
Ginseng	0	12.48 ± 16.38*	−7.57 ± 5.40*
Placebo	0	15.48 ± 5.17	15.89 ± 6.34

Data are mean ± SE values ($n = 40$ in ginseng group, $n = 42$ in placebo group).

*$P < .05$ by Student's t test for comparison with placebo group at the same interval.

Red ginseng extract does not improve facial elasticity, epidermal water content, erythema, and pigmentation in human skin

Baseline-adjusted ratios of Cutometer *CR2*, *CR5*, and *CR7* values between the treatment and control groups were not significantly different ($P > .05$, Mann-Whitney test), as shown in Table 3. Baseline-adjusted ratios of epidermal water content, erythema index, and melanin index revealed no differences between the two groups (data not shown).

Table 3. Percentage Change of Cutometer Values in Treatment and Placebo Groups

	Baseline	*12 weeks*	*24 weeks*
CR2			
Ginseng	0	−0.55 ± 3.08	2.77 ± 2.91
Placebo	0	21.61 ± 14.57	25.65 ± 19.33
CR5			
Ginseng	0	−6.02 ± 3.97	−7.85 ± 3.09
Placebo	0	2.66 ± 3.48	−4.46 ± 3.70
CR6			
Ginseng	0	−5.50 ± 4.04	−7.57 ± 4.22
Placebo	0	−2.80 ± 3.28	−12.17 ± 3.00
CR7			
Ginseng	0	−2.39 ± 3.80	−3.06 ± 3.31
Placebo	0	5.26 ± 4.00	1.28 ± 3.70

Data are mean ± SE values ($n = 40$ in ginseng group, $n = 42$ in placebo group).

No significant difference was observed between the treatment and control groups ($P > .05$, Mann-Whitney test).

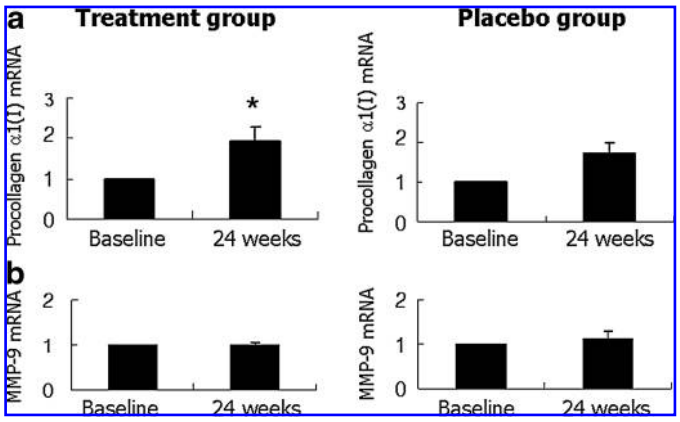

FIG. 1. Ginseng mixture treatment increases procollagen I mRNA expression in human skin. Total RNA was extracted from punch-biopsied skin samples. Relative (**a**) procollagen I mRNA ($n = 6$, treatment group; $n = 8$, placebo group) and (**b**) MMP-9 mRNA ($n = 8$, treatment group; $n = 8$, placebo group) were determined by real-time PCR analysis. Data are mean ± SE values. $^*P < .05$ by Wilcoxon signed rank test, compared with baseline.

Global assessment of clinical improvement in wrinkles, elasticity, and pigmentation demonstrated higher frequency of "slightly improved" and "improved" in the treatment group compared to the placebo group; however, the results were not significantly different between the two groups (data not shown).

Red ginseng extract increases type I procollagen gene expression while preventing MMP-9 gene induction in human skin

In the ginseng group ($n = 6$), type I procollagen mRNA levels increased significantly by 94% of baseline value ($P < .05$, Wilcoxon signed rank test). In the placebo group ($n = 8$), the gene expression increased by 72% but not significantly (Fig. 1a). MMP-9 gene expression did not reveal any changes after 24 weeks of administration in either the ginseng or placebo group (Fig. 1b). MMP-1 gene expression could not be determined because its gene expression was too low in the normal skin.

Red ginseng extract increases type I procollagen expression and fibrillin-1 fiber length in human skin

In the ginseng group, type I procollagen immunostaining was significantly increased to 162.0 ± 25.4% of baseline value, as shown by SP1.D8 stain (Fig. 2). In contrast, no change was seen in the placebo group. The total percentage area of fibrillin-1 was increased to 146.1 ± 16.7% of baseline value in the ginseng group and to 115.7 ± 20.3% in the placebo group; however, the changes were not significant in either group. The fibrillin-1 fiber length was elongated significantly to 171.1 ± 23.4% of baseline ($P < .05$) in the ginseng group and nonsignificantly to 118.6 ± 19.0% in the placebo group (Fig. 3). The total percentage area of tropoelastin immunostaining was increased to 138.4 ± 18.2% of baseline in the ginseng group and slightly decreased to 96.0 ± 8.7% in the placebo group; however, neither of the changes was significant. The changes in fiber length of tropoelastin exhibited the same trend as the total percentage area, with 143.1 ± 29.0% of baseline value in the ginseng group and 95.5 ± 7.8% in the placebo group, neither of which was significant (Fig. 4). The epidermal thickness did not reveal significant changes in either group.

DISCUSSION

P. ginseng, a perennial herb indigenous to Korea and China, has been used for traditional medicinal purposes since ancient times in Asia. The genus *Panax* derives its name from the Greek *pan* (all) and *akos* (healing), and true to its name, the herb has been known as the elixir of life in the Far East. Ginseng's known biological functions include immunostimulatory activity,[21,22] antigenotoxic properties,[23] adaptogenic effects,[24] antioxidant activity,[25,26] wound-healing effects,[27] anti-allergic effects,[28] and anti-aging effects.[29] However, the specific mechanisms underlying these biological activities have only begun to be revealed at the molecular and cellular levels because ginseng is a complex mixture of numerous potentially bioactive constituents, or ginsenosides (*e.g.*, saponins). Constituents of ginseng can be divided chemically into saponin and nonsaponin fractions. The saponins are further classified into three major groups:

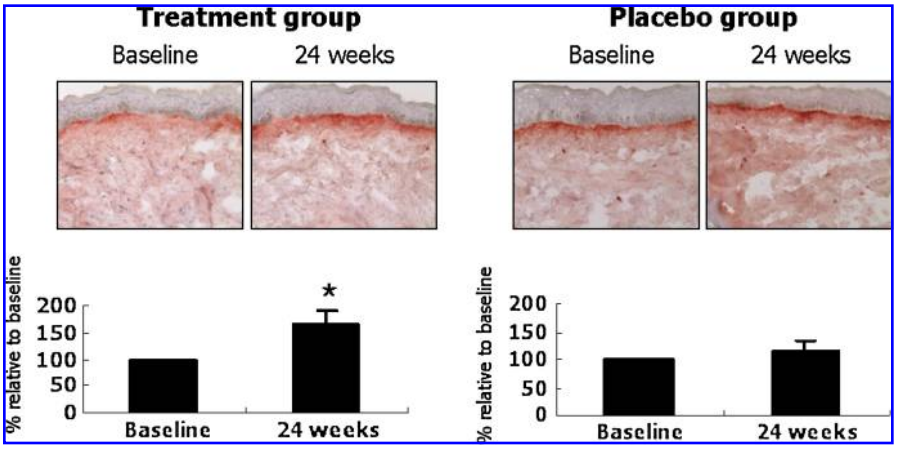

FIG. 2. Ginseng mixture treatment increases type I procollagen immunostaining in human facial skin. Immunohistochemistry for SP1.D8 was performed from punch-biopsied skin samples, and the degree of staining was visually graded by five dermatologists. Data are mean ± SE values ($n = 6$, treatment group; $n = 7$, placebo group). $^*P < .05$ by Wilcoxon signed rank test, compared with baseline.

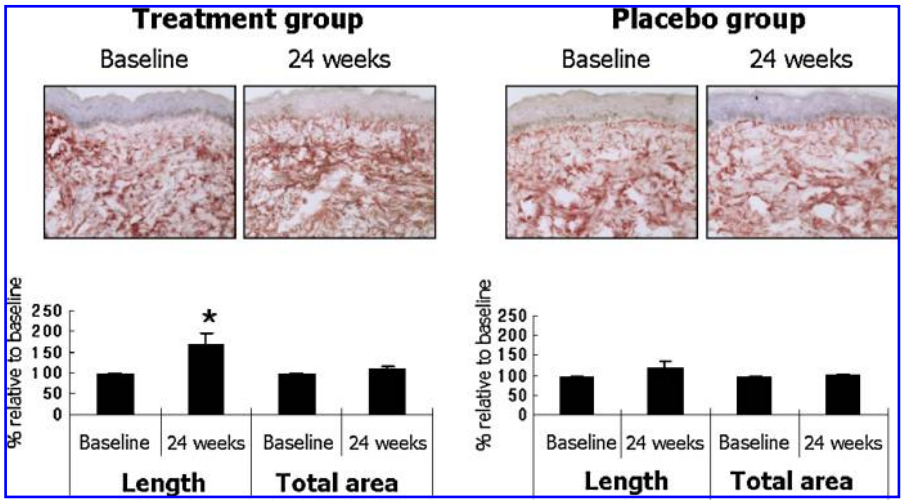

FIG. 3. Ginseng mixture treatment increases fibrillin-1 fiber length in the papillary dermis. Total percentage area and fiber length of fibrillin-1 were measured from the dermo-epidermal junction to 15 μm downward. The photographs are representative of six subjects in the ginseng group and seven subjects in the placebo group. Data are mean $\pm$ SE values. $^*P < .05$ by Wilcoxon signed rank test, compared with baseline.

the protopanaxadiol group, which includes Rb1, Rb2, Rc, Rd, and Rh2; the protopanaxatriol group, which includes Re, Rf, Rg1, Rg2, and Rh1; and oleanolic saponins, which include Ro.[30]

This study demonstrates, for the first time, objective evidence of a reduction in clinical facial wrinkles by long-term ingestion of ginseng extract, as shown by Visiometer values. Only the treatment group had significantly decreased depth of roughness (*R1*) and arithmetic average depth of roughness (*R5*) at 12 and 24 weeks of ingestion. The clinical improvement seen in facial wrinkles corresponds to the microscopic and biochemical increase in collagen synthesis. A recent study reported that *P. ginseng* extract induces human collagen production through the activation of COL1A2 promoter and activation of Smad signaling.[20] The results of our study are in line with this observation because real-time PCR demonstrated type I procollagen gene induction only in the treatment group. Furthermore, the immunohistochemical staining for type I procollagen showed increased staining at the dermo-epidermal junction only in the treatment group. The amount of collagen present in the dermis is the net result of its synthesis and degradation. Once the delicate homeostatic balance is shifted towards more synthesis or more breakdown, either sclerodermatous, *e.g.*, hard, skin or wrinkled, aged skin results, respectively. The previous study also reported inhibition of tumor necrosis factor-α-induced MMP-1 secretion. In the present study, for the lack of additional tissue, MMP-1 mRNA levels were not assessed; however, gene expressions of MMP-9, which further degrades collagen already fragmented by MMP-1, revealed no difference between the treatment and placebo groups.

Another possible mechanism by which ginseng induces collagen production is through its estrogen-like activity. Long-term ginseng ingestion was shown to be associated with estrogen-like activity, as evidenced by swollen and painful breasts in one study.[31] We previously demonstrated that topical application of 17β-estradiol increases type I procollagen synthesis by stimulating transforming growth factor-β signaling in aged human skin *in vivo*.[32] In addition, estrogen and retinoic acid are known to enhance hyaluronic acid synthesis and to prevent skin atrophy, dryness, and

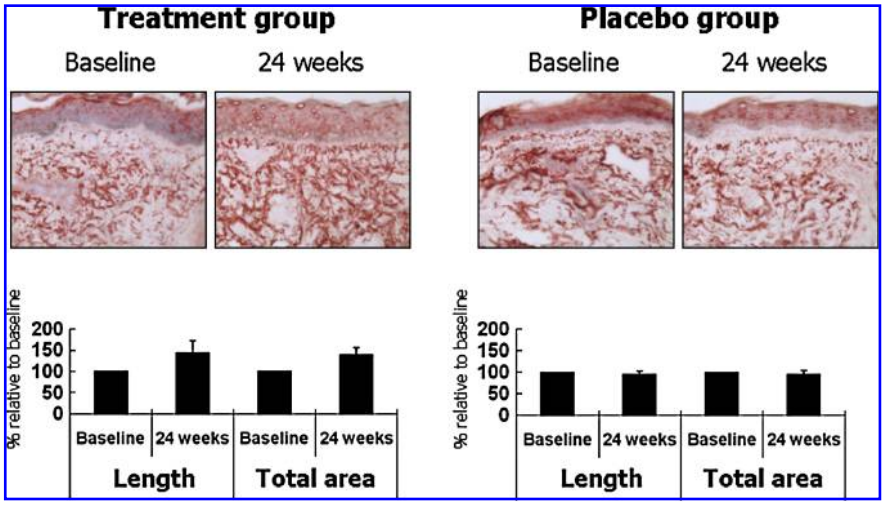

FIG. 4. Ginseng mixture treatment tends to increase tropoelastin fiber length and percentage area in the papillary dermis; however, the changes are not statistically significant (Wilcoxon's signed rank test). Total percentage area and fiber length of tropoelastin were measured from the dermo-epidermal junction to 50 μm downward. The photographs are representative of six subjects in the ginseng group and seven subjects in the placebo group. Data are mean $\pm$ SE values.

wrinkles in elderly women.[33] Because Compound K, a major metabolite of ginsenosides, increases hyaluronan in the mouse skin,[18] we speculate that the clinical improvement in the wrinkles may also be associated with increased hyaluronan as well as collagen.

Cutaneous elasticity was not shown to be changed by ginseng extract ingestion. Cutaneous elasticity is a composite result of the amount of elastic fiber and extracellular matrix-filling materials such as various proteoglycans. Although Cutometer readings did not demonstrate significant changes, the fiber length of fibrillin-1, a component of elastic fiber, was significantly elongated after long-term ginseng extract ingestion, hinting at potential delayed clinical benefit if supplemented for a long enough time.

There are few reports on the toxicity or side effects related to ginseng intake. In a 2-year human study, 10.5% of subjects who took ginseng at up to 15 g/day on a long-term basis experienced side effects such as hypertension, gastrointestinal disturbances, insomnia, and nervousness; other subjects who consumed extremely high (*e.g.*, >15 g/day) intake of ginseng showed symptoms of confusion and depression.[34] However, the validity of these observations is questionable because of the lack of placebo and control for other substance intake. The German Commission E recommended a daily intake of Asian ginseng of 1–2 g/day, containing 4–5% ginsenoside.[35] In the present study, the calculated amounts of daily ginseng intake and ginsenoside content are 2.72 g/day and 1.1%, respectively, which are not much higher than the recommended dosage. The fact that one subject from each group developed gastrointestinal discomfort implies that this adverse effect is not caused by the ginseng *per se* but rather by the physical burden of taking 10 hard capsules a day.

Ginseng has already been shown to protect human HaCaT keratinocytes from UVB-induced apoptosis by maintaining constant levels of Bcl-2.[36] The collagen-synthesizing activity of ginseng demonstrated through the present study adds evidence to the multifaceted anti-aging properties of ginseng. Much remains to be explored in this regard because ginseng is a complex molecular mixture of many biologically active substances. The limitation of this study includes the fact that the study substance consisted of not only ginseng but also two additional herbs. Corni fructus is commonly used in traditional Oriental medicine for treatment of diabetes and diabetic foot ulcer.[37] However, no anti-aging properties or effects on collagen synthesis have been reported for Corni fructus or Torilus fructus. The present study is the first long-term randomized controlled trial exploring the beneficial effects of red ginseng on photoaged human skin. Further controlled studies using pure red ginseng extracts are warranted to confirm the results of this study.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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