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Anti-wrinkle Effects of Black Snow, Sun, and Red Ginseng Water Extracts on Human Dermal Fibroblasts

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ABSTRACT

A variety of commercially processed ginseng products, including black snow ginseng (BSG), sun ginseng (SG), and red ginseng (RG) are currently available in the market. To evaluate the anti-wrinkle effects of *Panax ginseng* (BSG, SG, and RG) water extracts (PGWEs: BSGWE, SGWE, and RGWE), collagen synthesis and degradation abilities of PGWEs were assessed using human dermal fibroblasts. BSGWE, SGWE, and RGWE at the concentration of 50 μg/mL increased type-I procollagen synthesis by 82.7% ($p<0.001$), 26.9% ($p<0.01$), and 119.2% ($p<0.001$), respectively, in comparison to control cells. In comparison to control cells, treatments with 50 μg/mL of BSGWE, SGWE, and RGWE significantly ($p<0.001$) decreased the activity of matrix metalloproteinase-2 (MMP-2) and the mRNA and protein expression of MMP-1. PGWEs were found to promote collagen synthesis and inhibit its degradation in human dermal fibroblasts. Among PGWEs, RGWE showed the most effective anti-wrinkle effects.

Key words : Black snow ginseng, Collagen synthesis and degradation, MMP, Red ginseng, Sun ginseng

Introduction

Skin aging is a complex biological phenomenon. Biochemical changes associated with skin aging include reduced expression of type-I collagen mRNA, overexpression of matrix metalloproteinases (MMPs), and decreased synthesis of extracellular matrix (ECM). These alterations result in increased collagen degradation, and gradually lead to skin laxity, atrophy, wrinkles, dryness, and other clinical manifestations of skin aging [1,2].

Type-I collagen is the main component of ECM in the skin dermis, providing strength and maintaining the structure of this tissue layer. The quantity and quality of collagen are determined by the balance between its degradation and synthesis [3]. Increased expression of MMPs and decreased collagen synthesis are the major causes of wrinkle formation on the skin [4]. In particular, MMP-1 is the primary enzyme responsible for the degradation of dermal collagen during aging process of human skin [5,6]. Type-I collagen is the primary constituent of ECM, and its reduction is closely related to skin aging [7]. Therefore, analysis of the ability to affect MMP-1 expression levels and collagen production has become a primary screening process for the evaluation of potential anti-aging cosmetic ingredients [8].

Ginseng (*Panax ginseng* C., family Araliaceae) has been used for a long in the Far East, particularly in Korea and China as a well-known herbal medicine to maintain physical vitality [9]. Ginseng saponins, referred to as ginsenosides, are believed to play pharmacologically important roles, such as antioxidant, anti-apoptosis, and angiogenesis [10]. There are a variety of

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ginseng products, including white ginseng (WG), black ginseng (BG), red ginseng (RG), and sun ginseng (SG) that are commercially available.

Recently, we reported that *Panax ginseng* (BSG, SG, and RG) water extracts (PGWEs) have excellent melanogenesis-inhibiting effects via the inhibition of tyrosinase activity and melanogenesis-relevant gene expression [11,12]. In this study, the anti-wrinkle effects of PGWEs were evaluated by assessing the promotion of collagen synthesis and inhibition of mRNA and protein expression of MMP-1 in human dermal fibroblasts (HDFs).

Materials and Methods

1. Reagents and apparatus

Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin mixture (P/S), and Roswell Park Memorial Institute (RPMI)-1640 medium were obtained from Lonza Company (Cascade, MD, USA). Inverted microscope (CKX41, Olympus, Tokyo, Japan) and a CO_2 incubator (MCO-17AC, Sanyo electric, Osaka, Japan) were used to observe the cell growth and cell culture, respectively.

2. Preparation of ginseng extracts

BSG was obtained from Jong Beak Yoon Fermentation Red Ginseng Company (Daegu, Korea). The aqueous extract was lyophilized into powder form (BSGWE). SG water extract (SGWE) and RG water extract (RGWE) were obtained from Professor Jung Il Park (College of Pharmacy, Seoul National University, Seoul, Korea).

3. Cell culture

HDFs (13-16 passages) obtained from Amore Pacific Company (Osan, Korea) was used for sub-culturing. Cells were cultivated in DMEM supplemented with 10% FBS and 1% P/S in an incubator at 37°C and 5% CO_2.

4. UVA irradiation and sample treatment

HDFs were cultivated in the culture dish to approximately 80% confluency at 200 nm. After the removal of the medium, the cells were washed with PBS. To induce photoaging, the samples were subsequently treated at a cell density of 1.5×10^5 cells/mL in DMEM with 6.3 J/cm^2 UVA without FBS before irradiation. Cells were cultured for 24 h and used for MMP-2 activity assay and evaluation of MMP-1 expression.

5. Cell viability assay

HDFs were distributed in a 96-well plate (1×10^4 cells/well), and grown in the incubator at 37°C and 5% CO_2 for 24 h. Aliquots of PGWEs (200 μL) diluted with phenol red-free (PRF) DMEM to a range of concentrations (25-200 μg/mL) were added to the wells. The cells were grown in the incubator at 37°C and 5% CO_2 for 48 h. The cells were then placed in a medium containing 0.5 μg/mL of MTT and grown in the incubator at 37°C and 5% CO_2 for 3 h. After centrifugation of the plate for 10 min at 1000 rpm, the cells were pelleted and the medium was removed. DMSO (200 μL) was added to each well and the cells were resuspended for 15 min in a plate-shaker. Absorbance was measured using a plate reader at 540 nm (680, Bio-Rad, Tokyo, Japan).

6. Morphological observation of HDFs

HDFs were treated with 25, 50, and 100 μg/mL PGWEs, and the morphological changes of the cells were observed after 72 h of growth at 37°C and 5% CO_2. After incubation, the medium was changed and the cells were observed under an inverted microscope.

7. Procollagen assay

HDFs were treated with 25, 50, and 100 μg/mL of PGWEs. Following the treatment, the cells were centrifuged, supernatants were removed and analyzed using an enzyme immunoassay (EIA) kit (Takara, Japan). The absorbance was measured at 450 nm to calculate type-I procollagen protein yield, as previously described [13], with TGF-β1 (5 ng/mL) being used as a positive control.

HDFs were stabilized in DMEM containing 10% FBS and 1% P/S (DMEM-FBS-P/S), and incubated in a humidified 5% CO_2 atmosphere at 37°C for 24 h. Cells were harvested, divided into a 6-well plate at 500 μL volume/well containing 0.7×10^5 cells/well, and incubated for 24 h. After removing the medium, cells were washed twice with PBS. PRF-DMEM (1 mL) with 1% P/S was added to the cells and further incubated for 24 h. The cells were subsequently washed with PBS, and 3 mL of

PRF-DMEM containing PGWEs at the concentrations of 25, 50, and 100 μg/mL were added. The cells were incubated for 24 h, and the supernatants obtained after centrifugation at 12,000 rpm for 10 min were used in the subsequent procollagen protein quantitative analysis. The antibody-peroxidase-conjugate solution (100 μL) was added into the wells of a 96-well EIA kit with 20 μL of the 4-fold diluted samples. The kit was wrapped in a foil and incubated at 37°C for 3 h.

Following incubation, cells were washed 4 times with PBS, and 100 μL of the substrate solution was added, and the foil-wrapped kit was incubated at 25°C for 15 min. The stop solution (100 μL of 1 N H_2SO_4) was added and the absorbance was measured 3 times at 450 nm using a plate reader. The procollagen protein yield was separated from the protein content as previously described (Lowry et al., 1951) and quantified using BSA standards. Cell lysis buffer (80 μL of CelLytic B Cell Lysis Reagent, Sigma-Aldrich, St. Louis, MO, USA) was added to the plate, and the mixture frozen (at −20°C) and thawed 3 times before removing the mixture from the plate. The mixture was diluted 10 times and the protein content was quantified using the BSA standard curve.

8. MMP-2 activity

The cell culture solution was collected at specific volumes adjusted to ensure that the same amount of protein was loaded in each assay. The solution was mixed with an equal volume of sample buffer (2×) and incubated at 25°C for 10 min prior to being loaded onto 10% zymogram gel (Invitrogen, Carlsbad, CA, USA). Loaded proteins were separated by electrophoresis and incubated in the Novex zymogram renaturing buffer (1×) for 30 min. The buffer was transformed to Novex zymogram developing buffer (1×) and allowed to incubate at 25°C for 1 h. After changing to fresh developing buffer, the gel was incubated at 37°C for 30 min. After dyeing with the Simply blue safe stain (Invitrogen), the gel was washed with sterile distilled water and the white band was used to confirm the activity of MMP-2. MMP-2 activity was quantified using the image analyzer.

9. Reverse transcription-polymerase chain reaction (RT-PCR)

Levels of MMP-1 and β-actin mRNA were determined using RT-PCR method. The primer sequences used were as follows: MMP-1 forward 5′-CGA CTC TAG AAA CAC AAG AGC AAG A-3′, reverse 5′-AAG GTT AGC TTA CTG TCA CAC GCT T-3′; β-Actin forward 5′-ACC GTG AAA AGA TGA CCC AG-3′, reverse 5′-TAC GGA TGT CAA CGT CAC AC-3′. The expected sizes of PCR products for MMP-1 and β-actin were 237 and 248 base pairs, respectively. The PCR conditions used were as follows: 28 cycles of denaturation at 94°C for 60 s, annealing at 50°C for 60 s, and extension at 72°C for 60 s. PCR products were analyzed on 1.2% agarose gel, with β-actin used as an internal control to evaluate the relative expression of MMP-1.

10. Western blotting

Expression of MMP-1 protein was evaluated in cells lysates treated with 25, 50, and 100 μg/mL of PGWEs using western blotting with anti-MMP-1 antibody (ab 137332, 1 : 1000 dilution) (Abcam, Cambridge, UK) and β-actin as an internal control.

11. Statistical analysis

The differences among the groups were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan multiple range test using SPSS 21.0 for Windows (IBM, Armonk, NY, USA). The difference between the two groups was evaluated by Student's t-test. Statistical significance was set at $p < 0.05$.

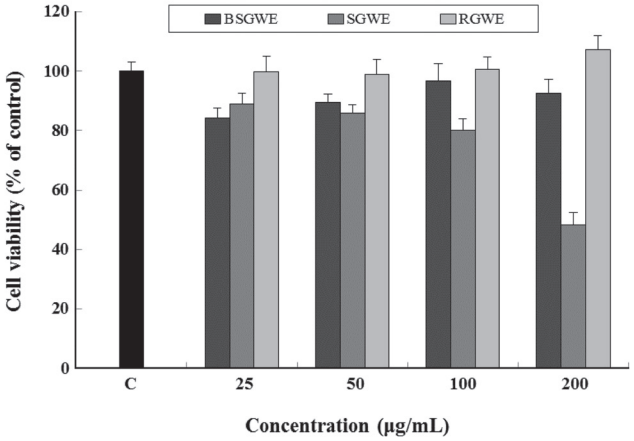

Fig. 1. Effect of ginseng extracts on cell viability. Viability of HDFs treated with test materials (0-200 μg/mL) for 48 h was analyzed by MTT assay. Values are presented as mean ± standard deviation (SD) for three independent measurements. C: control, BSGWE: black snow ginseng water extract, SGWE: sun ginseng water extract, RGWE: red ginseng water extract.

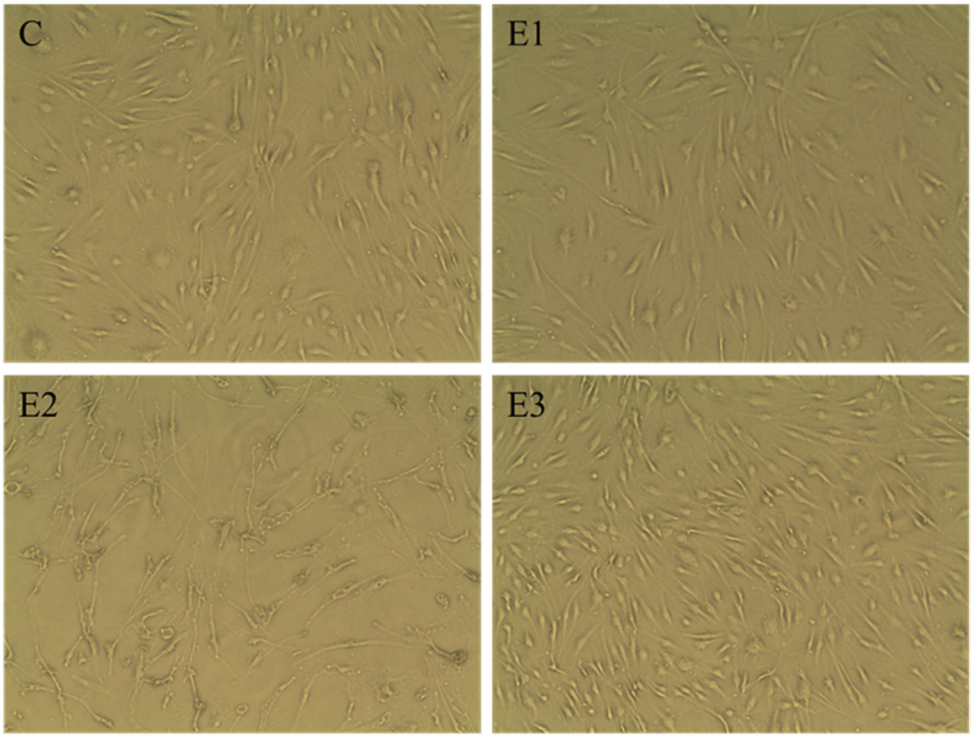

Fig. 2. Morphological changes in HDFs treated with ginseng extracts. Morphological changes in HDFs treated with 200 μg/mL BSGWE (E1), 200 μg/mL SGWE (E2), and 200 μg/mL RGWE (E3) for 48 h compared to untreated control (C). ×200 magnification.

Results

1. Effect of ginseng extracts on the viability of HDFs

BSGWE and RGWE did not reduce cell viability at concentrations between 25 μg/mL and 200 μg/mL; the cell viability was above 84.2%. However, the cell viability was reduced to 48.1% at 200 μg/mL SGWE. The maximum permissible level (MPL) for BSGWE and RGWE treatments to HDFs was over 200 μg/mL and MPL for SGWE was 100 μg/mL (Fig. 1).

2. Morphological observation of HDFs

Treatments of HDFs up to 200 μg/mL PGWEs except SGWE showed spindle-like features almost same as untreated control cells (E1, E3 and C in Fig. 2). Treatments of HDFs with 200 μg/mL SGWE resulted in cell death, showing shrunk and clumped features (E2 in Fig. 2).

3. Effect of ginseng extracts on type-I procollagen synthesis

In comparison to the control group, BSGWE, SGWE, and

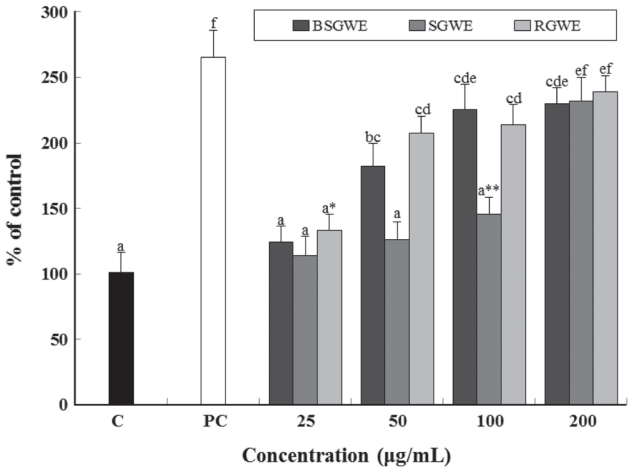

Fig. 3. Effect of ginseng extracts on collagen production in cultured HDFs. Collagen production in the cells treated with the indicated concentrations of test materials for 24 h was measured by EIA kit. Values are presented as mean ± standard deviation (SD) for three independent measurements. C: control, PC: 5 ng/mL TGF-β1, BSGWE: black snow ginseng water extract, SGWE: sun ginseng water extract, RGWE: red ginseng water extract. Values with different letters are significantly ($p<0.001$) different by ANOVA and Duncan multiple range tests. $^{**}p<0.01$, $^{*}p<0.05$ compared to the control.

RGWE treatments at 50 μg/mL significantly increased collagen production by 82.7% ($p<0.001$), 26.9% ($p<0.01$), and

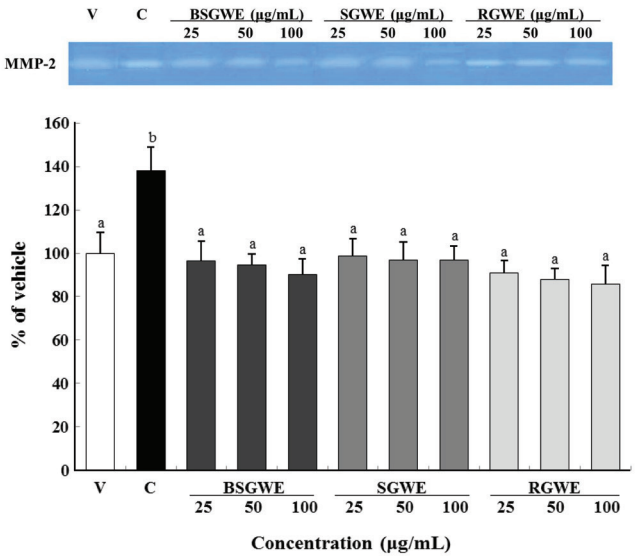

Fig. 4. Effect of ginseng extracts on MMP-2 activity in cultured HDFs. MMP-2 protein expression in the cells treated with the indicated concentrations of test materials for 48 h was analyzed by Zymography and quantified using the image analyzer. Values are presented as mean ± standard deviation (SD) for three independent measurements. V: vehicle, C: 6.3 J/cm^2 UVA-irradiation, BSGWE: black snow ginseng water extract, SGWE: sun ginseng water extract, RGWE: red ginseng water extract. Values with different letters are significantly ($p<0.001$) different by ANOVA and the Duncan multiple range test.

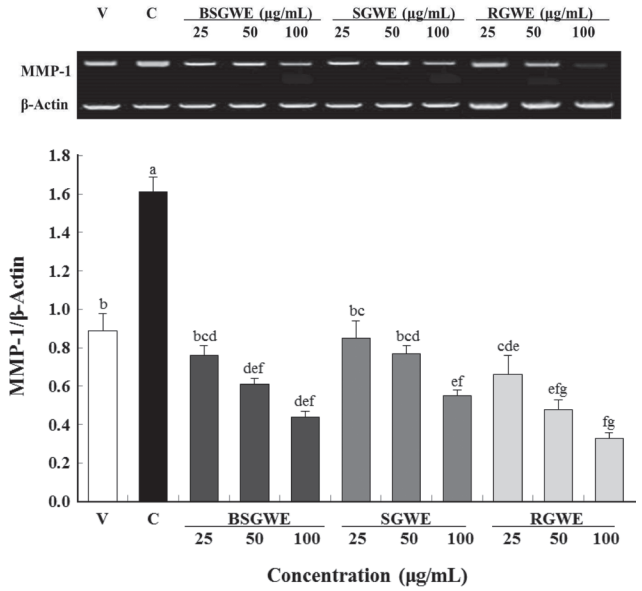

Fig. 5. Effect of ginseng extracts on MMP-1 mRNA expression in cultured HDFs. MMP-1 mRNA expression in cells treated with test materials (0-100 μg/mL) for 48 h was analyzed by RT-PCR and quantified using the image analyzer. Values are shown as mean ± standard deviation (SD) for three independent measurements. V: vehicle, C: 6.3 J/cm^2 UVA-irradiation, BSGWE: black snow ginseng water extract, SGWE: sun ginseng water extract, RGWE: red ginseng water extract. Values with different letters are significantly ($p<0.001$) different by ANOVA and the Duncan multiple range test.

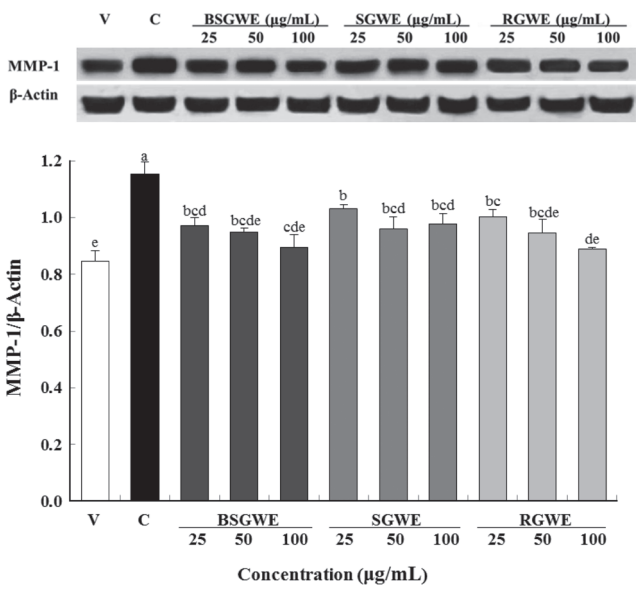

Fig. 6. Effect of ginseng extracts on MMP-1 protein expression in cultured HDFs. MMP-1 protein expression in cells treated with test materials (0-100 μg/mL) for 48 h was analyzed by western blotting. Values are shown as mean ± standard deviation (SD) for three independent measurements. V: vehicle, C: 6.3 J/cm^2 UVA-irradiation, BSGWE: black snow ginseng water extract, SGWE: sun ginseng water extract, RGWE: red ginseng water extract. Values with different letters are significantly ($p<0.001$) different by ANOVA and the Duncan multiple range test.

119.2% ($p<0.001$), respectively. Treatment with 5 ng/mL TGF-β1 (PC) also significantly increased collagen production by 165.0% ($p<0.001$) compared to control (Fig. 3).

4. Effect of ginseng extracts on MMP-2 activity

In comparison with the control group, BSGWE, SGWE, and RGWE treatments at 50 μg/mL significantly ($p<0.001$) decreased MMP-2 activity by 31.5%, 29.8%, and 36.3%, respectively (Fig. 4).

5. Effect of ginseng extracts on MMP-1 mRNA expression

In comparison to the control group, BSGWE, SGWE, and RGWE treatments at 50 μg/mL significantly ($p<0.001$) decreased MMP-1 mRNA expression by 62.1%, 52.2%, and 70.2%, respectively (Fig. 5).

6. Effect of ginseng extracts on MMP-1 protein expression

In comparison to the control group, BSGWE, SGWE, and

RGWE treatments at 50 μg/mL significantly ($p < 0.001$) decreased MMP-1 protein expression by 17.6%, 16.7%, and 23.0%, respectively (Fig. 6).

Discussion

Ginsenosides are the main active constituents of ginseng and are divided into 3 types according to the aglycone moieties: protopanaxadiol type (Rb1, Rb2, Rc, Rd, Rg3, and Rh2), protopanaxatriol type (Re, Rf, Rg1, Rg2, Rh1, and Rh4), and oleanolic acid (Ro) [14]. To date, more than 40 ginsenosides have been identified from ginseng [15]. Several types of ginseng products currently in common use differ with respect to the processing methods used in their production, including fresh ginseng (FG), WG, RG, BG, and SG [16]. RG is reported to be more pharmacologically active than WG [10].

Collagen and MMP-1 are well-known biological targets for the evaluation of the effectiveness of anti-wrinkle ingredients, with HDFs commonly used in the assays. Fibroblasts play an important role in the production of elastin, collagen, and substances such as glycosaminoglycans and proteoglycans [17]. In this study, PGWEs significantly ($p < 0.001$) increased collagen production in HDFs in the order RGWE > BSGWE > SGWE. SG extract were previously reported to increase collagen synthesis in HDFts [18]. RG extract has been reported to prevent UVB irradiation-induced skin photoaging [19]. The pharmacological and biological activities of steam-processed ginseng are greater than the activities of non-steamed ginseng. During the steaming process, relative proportions of major bioactive components, including ginsenosides, saponin, phenolics, and proteins, were altered as novel components are produced [20,21]. Several investigators have reported new ginsenosides (Rg1, Rg3, Rg5, Rh2, Rb1, and Rd) from RG that are not usually found in raw ginseng [22,23]. Steamed ginseng was suggested to exhibit a greater spectrum of pharmacological effects than WG [20,22,24]. According to Wang et al. [25], during the preparation of RG, particularly during steaming process, contents of ginsenosides Rg3 and Rh2 increased, and RG had more abundant ginsenosides Rb1 and Rd than WG and BG [26,27]. Rh2 would serve as an inducer of antioxidant enzymes [28]. Additionally, ginsenoside Rd increased type-I collagen mRNA expression in human dermal fibroblasts. Conversely, Rd reduced MMP-1 mRNA and protein level. In addition, Rd exhibited an increase in collagen protein level and a decrease in MMP-1 protein level in HDFs [29].

MMPs are enzymes that are directly responsible for the degradation of ECM components such as collagen and elastin. MMP-1, an interstitial collagenase, is a member of the MMP subfamily that specifically degrades the collagen triple helix [30]. In the present study, PGWEs significantly ($p < 0.001$) decreased MMP-2 activity, as well as MMP-1 mRNA and protein expression in human dermal fibroblasts. Rg1 decreased MMP-2 activity in RSC96 Schwann cells in a dose-dependent manner [31]. RG extract was reported to significantly ($p < 0.05$) decrease MMP-2 activity in SW480 colon cancer cells [32]. Additionally, SG extract was reported to decrease MMP-1 mRNA and protein expression in HDFs [16,18].

The results of the present study imply that PGWEs could increase collagen production directly and/or indirectly by suppressing MMP-1 mRNA and protein expression, as well as MMP-2 activity in HDFs. Among PGWEs, RGWE showed the most effective anti-wrinkle effects. It could be assumed that substantial differences exist between PGWEs, and that PGWEs might protect the skin against the formation of wrinkles; with steam processing in product preparation increasing the pharmacological effects. In conclusion, PGWEs were found to promote collagen synthesis and inhibit its degradation in HDFs. Therefore, PGWEs could be used as functional cosmetic agents for the prevention or alleviation of skin wrinkle formation.

References

1. Lupo MP. Cosmeceutical peptides. Dermatol Surg 2005;31: 832-836.
2. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, et al. Mechanisms of photoaging and chronological skin aging. Arch Dermatol 2002;138:1462-1470.
3. Dhalla AK, Hill MF, Singal PK. Role of oxidative stress in transition of hypertrophy to heart failure. J Am Coll Cardiol 1996;28:506-514.
4. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 1997;337:1419-1428.
5. Brennan M, Bhatti H, Nerusu KC, Bhagavathula N, Kang S, Fisher GJ, et al. Matrix metalloproteinase-1 is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin. Photochem Photobiol 2003;78:43-48.
6. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92:827-839.

7. Jung E, Lee J, Baek J, Jung K, Huh S, Kim S, et al. Effect of *Camellia japonica* oil on human type I procollagen production and skin barrier function. J Ethnopharmacol 2007;112:127-131.
8. Amano S, Ogura Y, Akutsu N, Matsunaga Y, Kadoya K, Adachi E, et al. Protective effect of matrix metalloproteinase inhibitors against epidermal basement membrane damage: skin equivalents partially mimic photoageing process. Br J Dermatol 2005; 153 (Suppl 2):37-46.
9. Yun TK. Brief introduction of Panax ginseng C.A. Meyer. J Korean Med Sci 2001;16:S3-S5.
10. Park JH, Cha HY, Seo JJ, Hong JT, Han K, Oh KW. Anxiolytic-like effects of ginseng in the elevated plus-maze model: comparison of red ginseng and sun ginseng. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:895-900.
11. Park EY, Rhie SJ, Kim YC. Comparison of antioxidant ability and whitening efficacy of black snow, snow, and red ginseng water extracts. J Invest Cosmetol 2017;13:193-199.
12. Park EY, Lee SH, Kim YC. Black snow, snow, and red ginseng water extracts downregulate the expression of genes related to melanogenesis in melan-a cells. J Invest Cosmetol 2017;13: 305-312.
13. Son ED, Choi GH, Kim H, Lee B, Chang IS, Hwang JS. Alpha-ketoglutarate stimulates procollagen production in cultured human dermal fibroblasts, and decreases UVB-induced wrinkle formation following topical application on the dorsal skin of hairless mice. Biol Pharm Bull 2007;30:1395-1399.
14. Chan TWD, But PPH, Cheng SW, Kwok IMY, Lau FW, Xu HX. Differentiation and authentication of *Panax ginseng*, *Panax quinquefolius*, and ginseng products by using HPLC/MS. Anal Chem 2000;72:1281-1287.
15. Yu H, Zhang C, Lu M, Sun F, Fu Y, Jin F. Purification and characterization of new special ginsenosidase hydrolyzing multiglycisides of protopanaxadiol ginsenosides, ginsenosidase type I. Chem Pharm Bull 2007;55:231-235.
16. Song KC, Chang TS, Lee H, Kim J, Park JH, Hwang GS. Processed *Panax ginseng*, sun ginseng increases type I collagen by regulating MMP-1 and TIMP-1 expression in human dermal fibroblasts. J Ginseng Res 2012;36:61-67.
17. Kondo S. The roles of cytokines in photoaging. J Dermatol Sci 2000;23:S30-S36.
18. Lee H, Lee JY, Song KC, Kim J, Park JH, Chun KH, et al. Protective effect of processed Panax ginseng, sun ginseng on UVB-irradiated human skin keratinocyte and human dermal fibroblast. J Ginseng Res 2012;36:68-77.
19. Hwang E, Sun ZW, Lee TH, Shin HS, Park SY, Lee DG, et al. Enzyme-processed Korean red ginseng extracts protects against skin damage induced by UVB irradiation in hairless mice. J Ginseng Res 2013;37:425-434.
20. Kim SN, Ha YW, Shin H, Son SH, Wu SJ, Kim YS. Simultaneous quantification of 14 ginsenosides in *Panax ginseng* C.A. Meyer (Korean red ginseng) by HPLC-ELSD and its application to quality control. J Pharm Biomed Anal 2007;45: 164-170.
21. Yun TK. Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. Mutat Res 2003;523-524: 63-74.
22. Baek NI, Kim DS, Lee YH, Park JD, Lee CB, Kim SI. Ginsenoside Rh4, a genuine dammarane glycoside from Korean red ginseng. Planta Med 1996;62:86-87.
23. Kasai R, Besso H, Tanaka O, Saruwatari Y, Fuwa T. Saponins of red ginseng. Chem Pharm Bull 1983;31:2120-2125.
24. Yun TK, Lee YS, Kwon KH, Choi KJ. Saponin contents and anticarcinogenic effects of ginseng depending on types and ages in mice. Acta Pharmacol Sin 1996;17:293-298.
25. Wang CZ, Aung HH, Ni M, Wu JA, Tong R, Wicks S, et al. Red American ginseng: ginsenoside constituents and antiproliferative activities of heat-processed *Panax quinquefolius* roots. Planta Med 2007;73:669-674.
26. Sun BS, Gu LJ, Fang ZM, Wang C, Wang Z, Lee MR, et al. Simultaneous quantification of 19 ginsenosides in black ginseng developed from *Panax ginseng* by HPLC-ELSD. J Pharm Biomed Anal 2009;50:15-22.
27. Kim WY, Kim JM, Han SB, Lee SK, Kim ND, Park MK, et al. Steaming of ginseng at high temperature enhances biological activity. J Nat Prod 2000;63:1702-1704.
28. Chang MS, Lee SG, Rho HM. Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenosides extracted from *Panax ginseng*. Phytother Res 1999; 13:641-644.
29. Kim WK, Song SY, Oh WK, Kaewsuwan S, Tran TL, Kim WS, et al. Wound-healing effect of ginsenoside Rd from leaves of *Panax ginseng* via cyclic AMP-dependent protein kinase pathway. Eur J Pharmacol 2013;702:285-293.
30. Kim J, Lee CW, Kim EK, Lee SJ, Park NH, Kim HS, et al. Inhibition effect of Gynura procumbens extract on UV-B-induced matrix-metalloproteinase expression in human dermal fibroblasts. J Ethnopharmacol 2011;137:427-433.
31. Lu MC, Lai TY, Hwang JM, Chen HT, Chang SH, Tsai FJ, et al. Proliferation- and migration-enhancing effects of ginseng and ginsenoside Rg1 through IGF-1- and FGF-2-signaling pathways on RSC96 Schwann cells. Cell Biochem Funct 2009; 27:186-192.
32. Seo EY, Kim WK. Red ginseng extract reduced metastasis of colon cancer cells in vitro and in vivo. J Ginseng Res 2011;35: 315-324.