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The Ginsenoside Derivative 20(*S*)-Protopanaxadiol Inhibits Solar Ultraviolet Light-Induced Matrix Metalloproteinase-1 Expression

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ABSTRACT

Ginsenosides are major pharmacologically active compounds present in ginseng (*Panax ginseng*). Among the ginsenosides, 20-*O*-*β*-*D*-glucopyranosyl-20(*S*)-protopanaxadiol (GPPD) and ginsenoside Rb1 (Rb1) have previously been reported to exhibit anti-wrinkle effects. In this study, 20(*S*)-protopanaxadiol (20(*S*)-PPD), an aglycone derivative of the Rb1 metabolite was investigated for its anti-wrinkle benefit and compared to GPPD and Rb1. The anti-wrinkle effect of 20(*S*)-PPD during solar UV light was investigated using a human skin equivalent model and human keratinocytes. 20(*S*)-PPD attenuated solar UV-induced matrix metalloproteinase (MMP)-1 expression to a greater extent than GPPD and Rb1. 20(*S*)-PPD treatment modulated MMP-1 mRNA expression and the transcriptional activity of activator protein (AP)-1, a major transcription factor of MMP-1. Two upstream signaling pathways for AP-1, the MEK1/2-ERK1/2-p90^{RSK} and MEK3/6-p38 pathways, were also suppressed. Taken together, these findings highlight the potential of 20(*S*)-PPD for further development as a preventative agent for sunlight-induced skin wrinkle. J. Cell. Biochem. 9999: 1–9, 2017.

KEY WORDS: SOLAR UV; 20(*S*)-PROTOPANAXADIOL; MMP-1; HUMAN SKIN EQUIVALENT

The skin is the largest organ of the human body and forms the largest selective interface with the surrounding environment [Fisher et al., 2002]. The aging of the skin is characterized by a progressive degradation of its components, and is influenced by several intrinsic and extrinsic factors [Makrantonaki and Zouboulis, 2007]. Irradiation by solar ultraviolet (UV) light is thought to be a major extrinsic factor, with an estimated 80% of facial skin aging arising from UV exposure [Uitto, 1997]. The primary symptoms of photoaged skin include wrinkles, dryness, laxity, rough pigmentation, and a leathery texture [Gilchrest and Yaar, 1992; Rittie and

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Fisher, 2002]. Solar UV is divided into a wavelength spectrum comprising three subgroups including UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm) [Brenneisen et al., 2002]. The Earth's ozone layer effectively prevents the penetration of UVC, although both UVA and UVB are able to reach the skin [Bruls et al., 1984; Matsumura and Ananthaswamy, 2004]. Solar UV which is consisted of about 94.5% UVA and 5.5% UVB is an actual cause of skin wrinkle [Bermudez et al., 2015]. Exposure to solar UV light in particular is associated with an increase in expression of matrix metalloproteinases (MMPs), which degrade skin collagen and are likely to play a role in wrinkle formation during the photoaging process [Fisher et al., 1997]. Repeated exposure to solar UV alters a number of biological pathways that reduce procollagen synthesis and break down the collagen-rich extracellular matrix (ECM) via the overexpression of MMPs [Fisher et al., 1997; Xu and Fisher, 2005]. Specific MMPs including MMP-1 (collagenase), MMP-2 (gelatinase), and MMP-9 (gelatinase) are primarily expressed in the human skin [Vu and Werb, 2000; Fisher et al., 2002; Rijken et al., 2005]. Of these, MMP-1 plays a pivotal role in the degradation of the types I and III collagens which are structural components of the ECM [Bruls et al., 1984; Fisher et al., 2002; Wenk et al., 2004]. Therefore, the attenuation of collagen degradation via the inhibition of MMP-1 expression to prevent wrinkle represents a potential therapeutic strategy for photoaging [Lim et al., 2014; Park et al., 2015].

MMP-1 is primarily regulated by transcription factors [Imokawa et al., 2015], of which AP-1 is a prominent example [Barchowsky et al., 2000; Tower et al., 2003; Lee et al., 2006]. AP-1 is a heterodimer that plays an important role in a number of inflammatory processes [Matthews et al., 2007]. The AP-1 transcription factor family includes JUN, JDP, FOS/FRA, ATF/CREB, and the MAF subfamilies [Ozanne et al., 2007], and its activation is dependent on dimer composition, transcript levels, post-translational modifications, and interactions with other proteins [Matthews et al., 2007]. UV-induced AP-1 activity is known to be mediated by the MAPK and Akt pathways [Rittie and Fisher, 2002], and the regulation of these pathways has been proposed as a good strategy to attenuate UV-induced MMP-1 expression [Lim et al., 2014; Huh et al., 2015; Roh et al., 2015].

Ginseng (*Panax ginseng*) has been widely used as a traditional medicinal plant for centuries [Tang and Eisenbrand, 1992; Yun, 2001], and has in recent years become popular as an ingredient in functional foods and cosmetics [Lim et al., 2015]. The pharmacological effects of ginseng have been reported to include anti-tumor activity [Mami et al., 1995], anti-diabetic efficacy [Lai et al., 2006], improvement in liver function [Huh et al., 1988], and anti-oxidant activity [Abdel-Wahhab and Ahmed, 2004]. Ginseng extract is also thought to aid in the prevention of skin wrinkle induced by UV irradiation [Kim et al., 2008; Kang et al., 2009; Lee et al., 2009; Yeom et al., 2010]. Ginsenosides are natural steroid glycosides and triterpene saponins that are the major pharmacologically active constituents of ginseng [Shibata et al., 1965; Yue et al., 2007; Christensen, 2008]. To date, more than 150 naturally derived ginsenosides have been isolated from various parts of the ginseng plant [Christensen, 2008]. Orally ingested ginseng extract is metabolically converted to various ginsenosides including ginsenoside Rb1 (Rb1, Fig. 1A), 20-*O*-*β*-*D*-glucopyranosyl-20(*S*)-protopanaxadiol (GPPD, Fig. 1B), and 20(*S*)-protopanaxadiol (20(*S*)-PPD, Fig. 1C) by intestinal bacteria [Hasegawa et al., 1996]. Rb1 has been shown to promote type I procollagen production in human dermal fibroblasts by activating the peroxisome proliferator-activated receptor-δ (PPARδ) and suppressing micro RNA-25 (miR-25) expression [Kwok et al., 2012a]. We have previously demonstrated that GPPD inhibits UV-induced MMP-1 expression via suppression of the AMPK-mediated mTOR pathway [Shin et al., 2014]. However, the MMP-1 suppressing activity and mechanism responsible for the effect of 20(*S*)-PPD, the final metabolite of ginsenoside Rb1 in the human body, remains to be investigated.

In this study, we elucidated the anti-wrinkle effect of 20(*S*)-PPD on solar UV-induced MMP-1 expression in a human skin equivalent

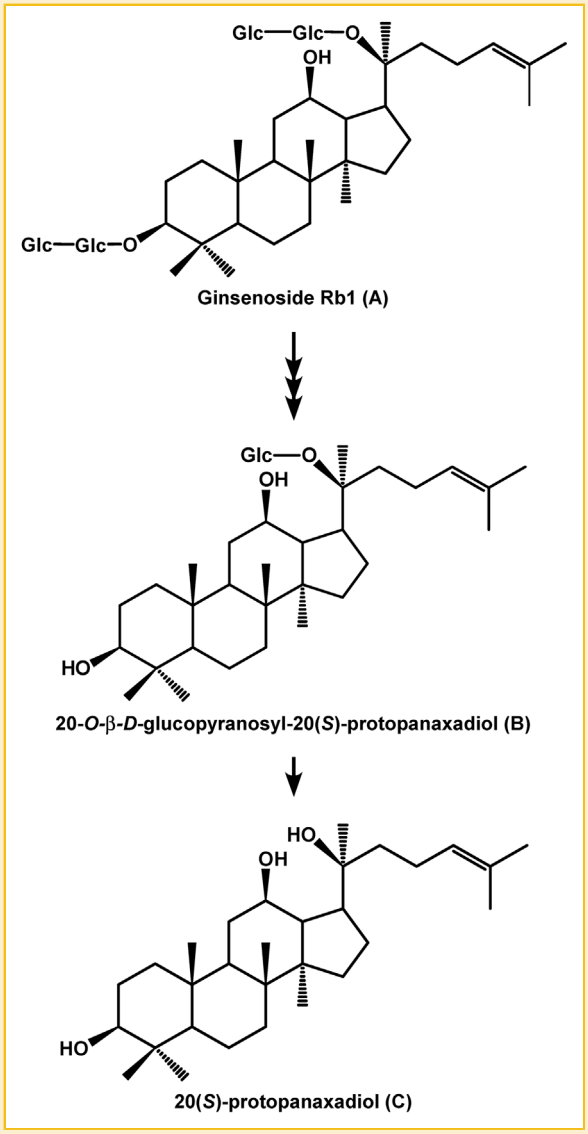

Fig. 1. Chemical structure and bioconversion pathway for ginsenoside Rb1 (Rb1), 20-*O*-*β*-*D*-glucopyranosyl-20(*S*)-protopanaxadiol (GPPD) GPPD, and 20(*S*)-protopanaxadiol (20(*S*)-PPD).

model and human keratinocytes (HaCaT). We also sought to investigate the molecular mechanisms responsible for the prevention of solar UV-induced MMP-1 expression.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

20(*S*)-PPD and GPPD were provided by the Ambo Institute (Seoul, Republic of Korea). Rb1 was provided by Nanjing Zelang Medical Technology Co., Ltd. (Jiangsu, China). Dulbecco's modified Eagle medium (DMEM) was purchased from Hyclone (Logan, UT) and fetal bovine serum (FBS) was purchased from Sigma–Aldrich (St. Louis, MO). Penicillin–streptomycin solution was purchased from Mediatech, Inc. (Manassas, VA). The CellTiter 96® AQueous One Solution Cell Proliferation Assay was purchased from Promega Corporation (Madison, WI). The MMP-1 antibody was obtained from R&D Systems Inc. (Minneapolis, MN). Antibodies against phosphorylated extracellular-signal regulated kinase (ERK) 1/2 (Thr202/Tyr204), total ERK1/2, and total c-Jun N-terminal kinase (JNK) 1/2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other antibodies were purchased from Cell Signaling Biotechnology (Beverly, MA). The lentiviral expression vectors including pGF-AP1-mCMV-EF1-Puro (System Biosciences, CA), and packaging vectors including pMD2.0G and psPAX, were purchased from Addgene Inc. (Cambridge, MA). pGF-MMP-1-mCMV-EF1-puro vector was generously provided by Dr. Sung-Keun Jung (Korea Food Research Institute, Seongnam, Republic of Korea) [Kim et al., 2009].

CELL CULTURE AND TREATMENTS

HaCaT cells were purchased from CLS Cell Lines Services GmbH (Heiderberg, Germany). HaCaT cells were cultured in DMEM with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin at 37°C and 5% CO_2.

SOLAR UV IRRADIATION

HaCaT cells were exposed to solar UV at 25 kJ/m^2 in serum free media. Human skin equivalent sample were exposed to solar UV at 30 kJ/m^2 in serum free media. The solar UV light source was purchased from Q-Lab Corporation (Cleveland, OH). The UVA-340 lamps purchased produce an optimal simulation of sunlight in the critical short wavelength region from 365 nm down to the solar cut off of 295 nm with a peak emission of 340 nm. The percentage of UVA and UVB produced by the UVA-340 lamps was measured with a UV meter at 94.5% and 5.5%, respectively [Kim et al., 2016].

CELL VIABILITY

Cell cytotoxicity was measured using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. HaCaT cells were cultured in 96-well plates at a density of 10×10^4 cells/well and incubated in DMEM-10% FBS contacting penicillin/streptomycin at 37°C and 5% CO_2. Cells were starved in serum-free DMEM for 24 h prior to treatment with various concentrations of the test compounds for 1 h, followed by solar UV irradiation at 25 kJ/m^2. The cells were incubated for 48 h after solar UV irradiation at 37°C and 5% CO_2. Cells were then treated with MTS solution activated with PMS solution for 2 h. The absorbance at 490 nm was measured using a microplate reader (Molecular Devices, CA).

WESTERN BLOT

For MMP-1, HaCaT cells were cultured for 48 h, and then incubated in serum-free DMEM for 24 h, before treatment with the indicated concentrations of 20(*S*)-PPD, GPPD, or Rb1 (0.25, 0.5 and 1 μg/mL) for 1 h, followed by solar UV (25 kJ/m^2) irradiation. The media was harvested on ice, and then centrifuged at 18,620*g* for 10 min. For other protein extractions, cell lysates were prepared using cell lysis buffer (50 mM tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride 1 mM Na_3VO_4, 1 mM dithiothreitol). Protein concentrations were measured using a protein assay reagent kit as described by the manufacturer. The proteins were separated electrophoretically using a 10% SDS–polyacrylamide gel and transferred onto an Immobilon P membrane (Merck Millipore). The membrane was blocked in 5% fat-free milk for 1 h, and then incubated with the specific primary antibody at 4°C overnight. Protein bands were visualized using a chemiluminescence detection kit (GE Healthcare, London, UK) after hybridization with the HRP-conjugated secondary antibody (Santa Cruz). Western blot data were quantified using the program Image J (NIH, WA).

GELATIN ZYMOGRAPHY

Gelatin zymography was performed in 12% polyacrylamide gels in the presence of gelatin (0.1% w/v) as a substrate for MMP-2. The protein samples were mixed with loading buffer (10% SDS, 25% glycerol, 0.25 M pH 6.8 Tris buffer and 0.1% bromophenol blue), and then run on 12% SDS–PAGE gels without denaturation. The gels were then washed with renaturing buffer (Life Technologies) for 1 h at room temperature and incubated for 24 h at 37°C in developing buffer (Life Technologies). After the enzyme reaction, the gels were stained with 0.5% Coomassie brilliant blue in 10% acetic acid.

REAL-TIME QUANTITATIVE PCR

HaCaT cells were treated with 20(*S*)-PPD for 24 h and harvested in RNAiso Plus (Takara Bio Inc., Shiga, Japan). RNA was quantified using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). After RT with oligo-dT primers using a PrimeScript™ 1st strand cDNA synthesis kit (Takara Bio Inc.), Real-time quantitative RT-PCR was performed using IQ SYBR (Bio-Rad Laboratories) and 2 μL of cDNA in triplicate with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control. Before PCR amplification, the primers were denatured at 95°C for 3 min. Amplification consisted of 44 cycles at 95°C for 10 s, 60°C for 30 s, and 72°C for 30. PCR was performed using a CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). cDNA was probed by the following primer: MMP-1 forward (5′-CCC CAA AAG CGT GTG ACA GTA-3′); MMP-1 reverse (5′-GGT AGA AGG GAT TTG TGC G-3′); GAPDH forward (5′-GAG TCA ACG GAT TTG GTC GT-3′); GAPDH reverse (5′-TTG ATT TTG GAG GGA TCT CG-3′).

LUCIFERASE REPORTER GENE ASSAY

pGF-AP1-mCMV-EF1-Puro vector or pGF-MMP-1-mCMV-EF1-puro vector and the packaging vectors (pMD2.0G and psPAX)

were transfected into HEK293T cells using jetPEI following the manufacturer's instructions. The transfection medium was changed 24 h after transfection and the cells were then cultured for a further 36 h. The viral particles were harvested by filtration using a 0.45 μm syringe filter, then combined with 8 μg/mL polybrene (EMD Millipore, Darmstadt, Germany) and infected into 60% confluent HaCaT cells overnight. The cell culture medium was replaced with fresh complete growth medium for 24 h before the cells underwent puromycin selection (Sigma, MO, 2 μg/mL) over 36 h. HaCaT cells that had successfully transduced the vectors were cultured for 48 h and then starved in serum-free DMEM for 24 h. After starvation, the cells were treated with various concentrations of 20(*S*)-PPD for 1 h, followed by 25 kJ/m^2 solar UV irradiation. Cell extracts were prepared after either 8 h (AP-1) or 24 h (MMP-1) with reporter lysis buffer (Promega). MMP-1 transcription and AP-1 transactivity in the HaCaT cells was determined using a luciferase assay kit (Promega), as described by the manufacturers.

PREPARATION OF HUMAN SKIN EQUIVALENT

Neoderm®-ED which is a human skin equivalent model was purchased from TEGO Science (Seoul, Korea). Briefly, human dermal fibroblasts (HDFs) were cultured in the collagen matrix for 1 day before keratinocytes were seeded on top of the collagen matrix and co-cultured for 4 days. The keratinocytes were then lifted and the HDF block exposed to the air. 20(*S*)-PPD was treated for 1 h, 7 days after the air-lift and the cells were irradiated with 30 kJ/m^2 solar UV twice daily for 2 days. Incubation settings were 37°C in an atmosphere of 5% CO_2.

MASSON'S TRICHROME STAINING

To analyze collagen in the dermis, Masson's trichrome staining was performed. Human skin equivalent samples were fixed with 10% neutral-buffered formalin, and embedded in paraffin. Serial sections (4 μm) were mounted onto slides and after deparaffinizing, the sections were stained with hematoxylin for 5 min. The slides were then washed and stained in biebrich scarlet and acid fuchsin before submersion in phosphomolybdic-phosphotungstic acid for 10 min and aniline blue for 5 min to stain the collagen. Slides were then washed and incubated in 1% acetic acid for 15 min, before dehydration and washing. Skin sections were examined at 400× magnification using an Olympus AX70 light microscope (Tokyo, Japan).

STATISTICAL ANALYSIS

Differences between the control and the solar UV-irradiated control were assessed with Student's *t*-test. To compare the difference between the solar UV-exposed groups, one-way ANOVA was used with Duncan's multiple range test. The data were statistically analyzed with IBM SPSS Statistics Ver. 23.0 (IBM Co., Armonk, NY) and *P*-values of less than 0.05 were considered statistically significant.

RESULTS

20(*S*)-PPD INHIBITS SOLAR UV-INDUCED MMP-1 PROTEIN EXPRESSION IN HaCaT CELLS

In our previous study, Rb1 and GPPD exhibited anti-wrinkle effects [Kwok et al., 2012b; Shin et al., 2014]. Thus, to verify the

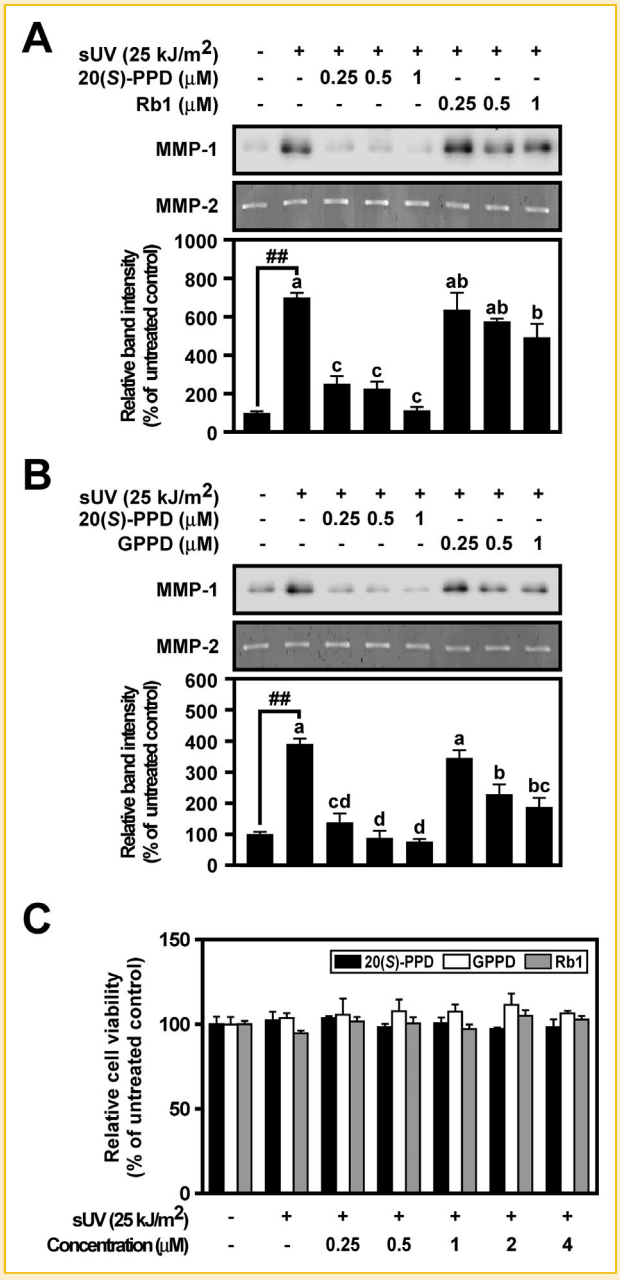

Fig. 2. Effect of 20(*S*)-PPD on solar ultraviolet (UV)-induced matrix metalloproteinase (MMP)-1 protein expression in human keratinocytes (HaCaT). (A and B) Effect of 20(*S*)-PPD on MMP-1 expression compared to ginsenoside Rb1 (A) and GPPD (B). HaCaT cells were pretreated with 20(*S*)-PPD, Rb1 or GPPD at the indicated concentrations for 1 h, and then further treated with 25 kJ/m^2 solar UV for 48 h at 37°C. Protein expression was then analyzed by Western blot assay. MMP-2 was used as a loading control as determined by gelatin zymography. (C) Cell viability following 20(*S*)-PPD, GPPD, and Rb1 treatment as measured by the CellTiter 96® AQueous One Solution Cell Proliferation Assay. Cells were pretreated with 20(*S*)-PPD, GPPD, or Rb1 at the indicated concentrations for 1 h, and then further treated with 25 kJ/m^2 solar UV light for 48 h at 37°C. Data (n = 3) represent the mean values ± SEM. Means with letters (a–c) are significantly different from each other at $P < 0.05$ ($^{\#\#}P < 0.01$), relative to the control cells.

MMP-1 suppressing activity of 20(*S*)-PPD (the primary metabolite of Rb1 and GPPD), we evaluated the inhibitory effects on solar UV-induced MMP-1 protein expression in HaCaT cells compared to Rb1 (Fig. 1A) or GPPD (Fig. 1B). MTS assay results showed that 20(*S*)-PPD, Rb1 and GPPD did not exhibit cytotoxicity until 4 μM concentrations (Fig. 2C). Although Rb1 and GPPD revealed inhibitory effect on solar UV-induced MMP-1 protein expression in HaCaT cells as we reported, 20(*S*)-PPD represented the strongest activity against MMP-1 protein level (Fig. 2A and B).

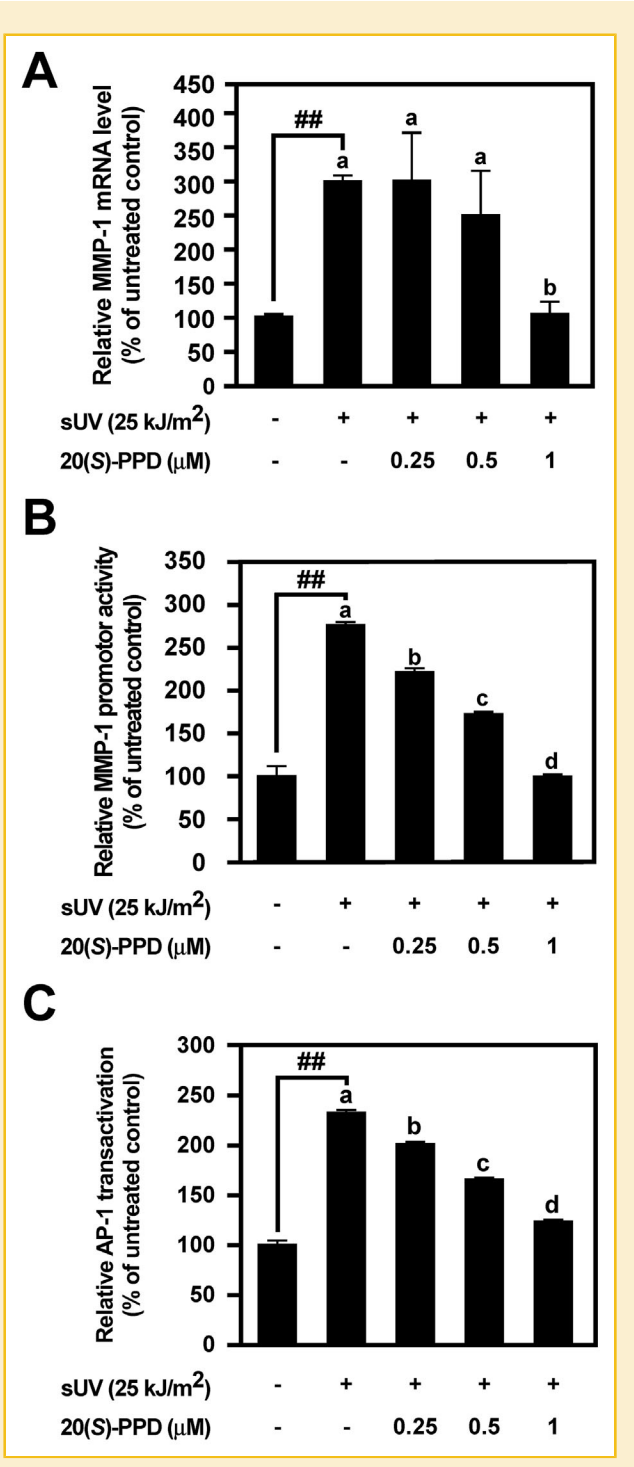

Fig. 3. Continued.

20(*S*)-PPD INHIBITS SOLAR UV-INDUCED MMP-1 mRNA AND PROMOTER ACTIVITY BY SUPPRESSING AP-1 TRANSACTIVATION

To verify how solar UV-induced MMP-1 expression is regulated by 20(*S*)-PPD treatment, we investigated MMP-1 transcriptional activity. 20(*S*)-PPD treatment was observed to downregulate solar UV-induced MMP-1 mRNA transcript levels (Fig. 3A), which occurred in a dose-dependent manner (Fig. 3B). We found that the exposure of solar UV 3-fold increased the MMP-1 mRNA transcript levels, and 1 μM of 20(*S*)-PPD lowered the MMP-1 mRNA to similar level with control group. solar UV irradiation stimulated AP-1, a major transcription factor of MMP-1 [Rittie and Fisher, 2002]. Using a luciferase reporter gene assay, 20(*S*)-PPD was also observed to dose-dependently inhibit solar UV-induced AP-1 transactivation in HaCaT cells (Fig. 3C), indicating that 20(*S*)-PPD suppresses solar UV-induced MMP-1 transcription by suppressing AP-1 activity in HaCaT cells.

20(*S*)-PPD SUPPRESSES SOLAR UV-INDUCED MEK1/2-ERK-p90^{RSK} AND MEK3/6-p38 PATHWAYS IN HaCaT CELLS

We previously observed that the regulation of mitogen-activated protein kinases (MAPKs) and the Akt signaling pathway can play a pivotal role in suppressing AP-1 transactivation [Rittie and Fisher, 2002; Dong et al., 2008]. Therefore, we sought to examine the effect of 20(*S*)-PPD on solar UV-induced MAPK and Akt signaling. The phosphorylation leves of MAPKs and Akt remarkably increased by solar UV irradiation, and 20(*S*)-PPD suppressed solar UV-induced phosphorylation of MEK1/2-ERK1/2-p90^{RSK} and MEK3/6-p38 in a dose-dependent manner (Fig. 4A and B). However, 20(*S*)-PPD did not modulate the MKK4-JNK1/2 and Akt-p70^{S6K} signaling pathways (Fig. 4C and D), indicating that 20(*S*)-PPD suppresses solar UV-induced MMP-1 transcription by suppressing AP-1 activity via regulation of the MEK1/2-ERK1/2-p90^{RSK} and MEK3/6-p38 pathways.

Fig. 3. Effect of 20(*S*)-PPD on solar UV-induced MMP-1 gene transcription, gene promotor activity, and activator protein (AP)-1 transactivation in HaCaT cells. (A) MMP-1 mRNA expression was determined by real-time quantitative PCR. Cells were pretreated with 20(*S*)-PPD for 1 h, and then further treated with 25 kJ/m^2 solar UV for 24 h at 37°C. (B) Effect of 20(*S*)-PPD on solar UV-induced MMP-1 promotor activity. HaCaT cells transduced with an MMP-1 promoter reporter plasmid were pretreated with 20(*S*)-PPD at the indicated concentrations for 1 h, before exposure to 25 kJ/m^2 solar UV at 37°C. Cell extracts were collected after 8 h. MMP-1 promotor activity was measured using a luciferase reporter gene assay. (C) AP-1 transactivation activity induced by 20(*S*)-PPD. HaCaT transduced with an AP-1 reporter plasmid were pretreated with 20(*S*)-PPD at the indicated concentrations for 1 h, and then further treated with 25 kJ/m^2 solar UV. Cell extracts were collected after 4 h. AP-1 transactivation was measured using a luciferase reporter gene assay. Data (n = 3) represent the mean values ± SEM. Means with letters (a–d) in a graph are significantly different from each other at $P < 0.05$ ($^{\#\#}P < 0.01$_, relative to the control cells.

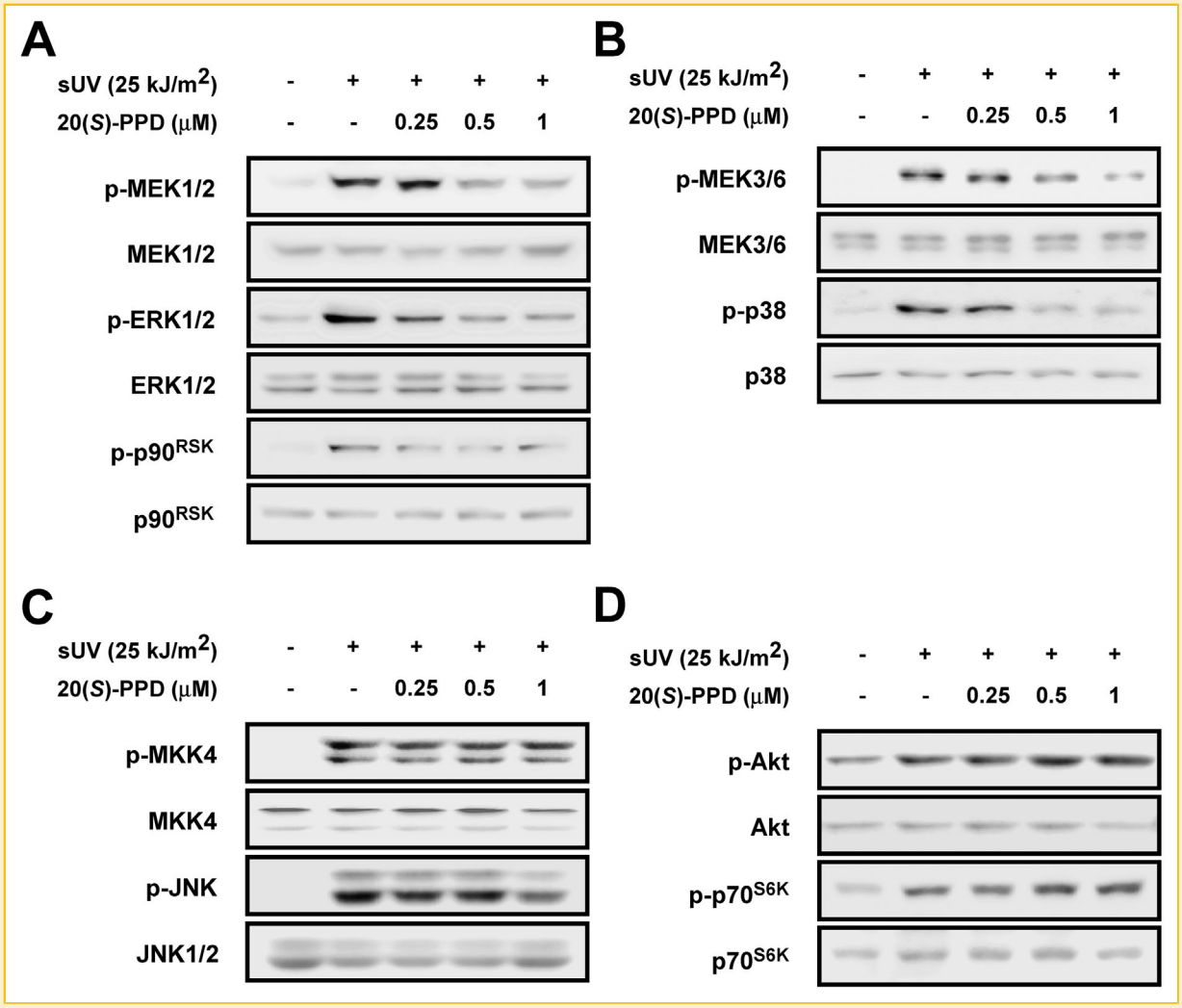

Fig. 4. Inhibitory effect of 20(*S*)-PPD on solar UV-induced signaling pathways. (A) Effect of 20(*S*)-PPD on solar UV-induced phosphorylation of the MEK1/2-ERK1/2-p90^{RSK} signaling pathway. (B) Effect of 20(*S*)-PPD on solar UV-induced phosphorylation of the MEK3/6-p38 signaling pathway. (C and D) Effect of 20(*S*)-PPD on solar UV-induced phosphorylation of the MKK4-JNK1/2 and Akt-p70^{S6K} signaling pathways. Following 20(*S*)-PPD pretreatment and solar UV irradiation, the cells were lysed as described in the Materials and Methods section. Phosphorylated and total protein expression was determined by Western blot with the indicated antibodies.

20(*S*)-PPD SUPPRESSES SOLAR UV-INDUCED MMP-1 EXPRESSION AND COLLAGEN DEGRADATION IN HUMAN SKIN EQUIVALENT

To verify the physiological relevance of the MMP-1 suppressing effect of 20(*S*)-PPD, we examined the effect of 20(*S*)-PPD on MMP-1 expression and collagen fibers in a human skin equivalent model as described in Figure 5A. After 2 days of solar UV irradiation, cell extracts were collected and paraffin-embedded 3D human skin tissues were sectioned. The embedded sections were treated with Masson's trichrome staining as described in the Materials and Methods section. Similar with Figure 2, solar UV increased MMP-1 expression in human skin equivalent model. And, 20(*S*)-PPD inhibited solar UV-induced MMP-1 expression in a dose-dependent manner (Fig. 5B). Additionally, we evaluated the collagen content in this model. As we expected, the collagen level in solar UV-treated human skin equivalent tissue was significantly reduced and 20(*S*)-PPD recovered collagen content in the section (Fig. 5C and D).

DISCUSSION

This study reveals the possible of 20(*S*)-PPD for development skin whitening agent. Especially, we found that 20(*S*)-PPD suppressed MMP-1 protein expression under non-cytotoxic concentration (Fig. 2). Notably, this activity was shown in human skin equivalent model as described in Figure 5. Although the chemical safety and clinical test are obviously necessary for expanding this finding to develop skin whitening agent, there is no remarkable morphological changes of the sample treated group of human skin equivalent model with MMP-1 suppression by 20(*S*)-PPD.

GPPD is a metabolite of Rb1 and has properties that make it attractive for use in cosmetics [Kim, 2015]. To increase the yield of GPPD that can be extracted from ginseng, red ginseng is produced by repeatedly steaming and drying ginseng in line with traditional methods [Lee et al., 2009]. In recent years, a number of technologies

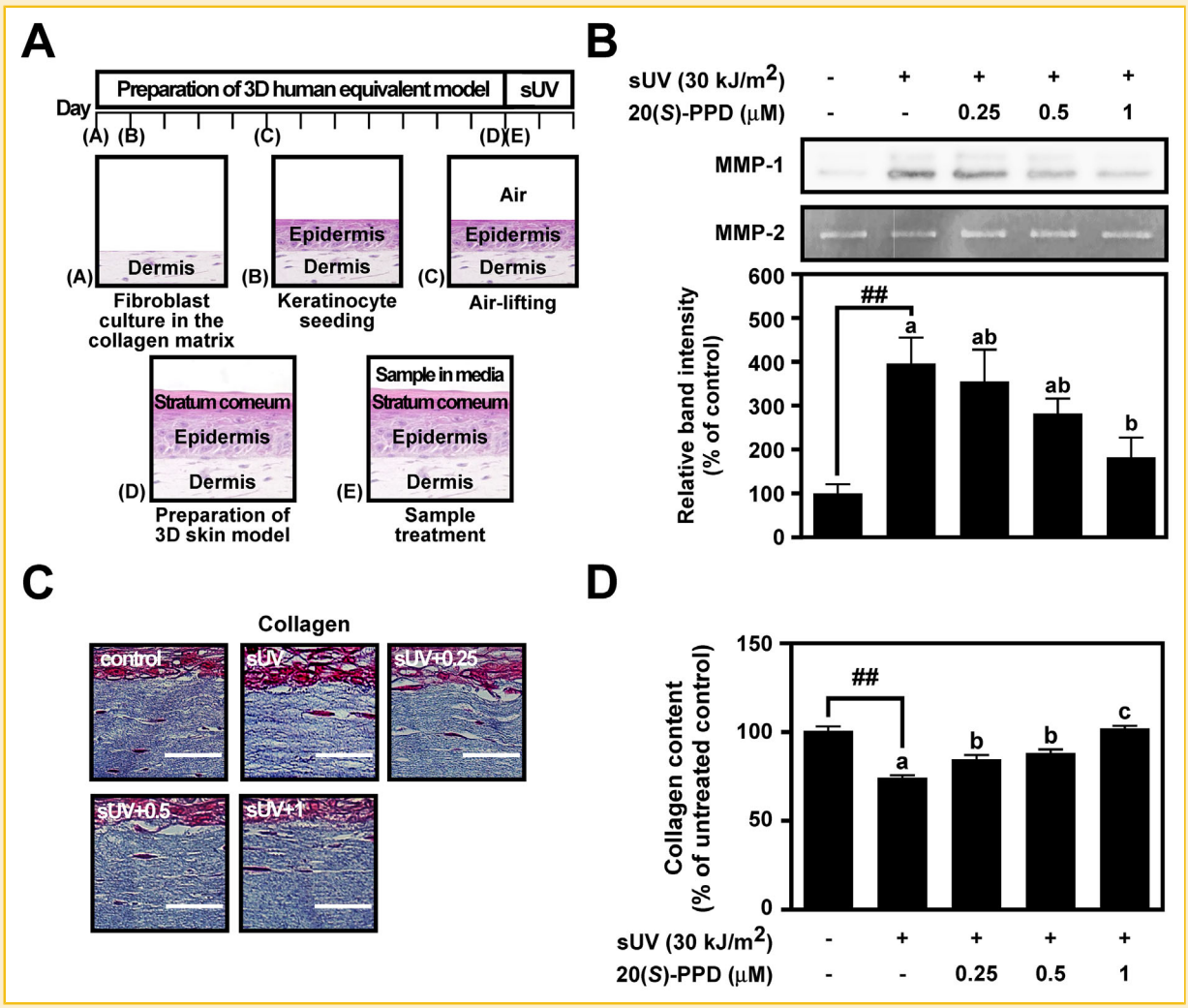

Fig. 5. 20(*S*)-PPD inhibits solar UV-induced collagen degradation and MMP-1 expression in a human skin equivalent model. (A) Schematic diagram of the experiment. The experiment was performed as described in the Materials and Methods section. (B) The effect of 20(*S*)-PPD on MMP-1 protein expression was determined by Western blot. (C and D) Masson's trichrome staining for the visualization of collagen fibers (C). Collagen fibers appear blue (D). Collagen content was quantified using the Image J software (NIH, WA). Data (n = 7) represent the mean values ± SEM. Means with letters (a–c) in a graph are significantly different from each other at $P < 0.05$ ($^{\#\#}P < 0.01$) relative to control cells.

for producing GPPD through bioconversion approaches have been developed [Yoo et al., 2011]. GPPD has a single sugar moiety and 20(*S*)-PPD is an aglycone of GPPD [Lim et al., 2015]. When making GPPD from ginseng extract or Rb1, it is necessary to leave the specific sugar moiety intact [Lu et al., 2009]. However, when producing 20(*S*)-PPD, each sugar should be removed [Yoo et al., 2011]. This also creates economic advantages, as 20(*S*)-PPD can be obtained with a much higher yield at a lower price. In the present study, we have shown that 20(*S*)-PPD exhibits more potent inhibitory effects against solar UV-induced MMP-1 expression than GPPD. Based on clinical experience, compounds with a molecular weight (MW) of less than 500 Daltons are considered viable for skin absorption. [Bos and Meinardi, 2000; Jakasa et al., 2007]. Because the MW of 20(*S*)-PPD is approximatively 460 g/mol, it seems likely that it may pass the corneal layer in contrast to other ginsenosides.

Sun exposure is a major factor that can accelerate the premature skin aging process, and this phenomenon is called photoaging [Chung et al., 2002; Wenk et al., 2004]. Skin photoaging is a process of senescence and is commonly associated with representative symptoms such as wrinkles, sagging, and laxity [Jenkins, 2002]. Solar UV irradiation results in the overexpression of MMP-1, which plays a major role in wrinkle via the disruption of tissue integrity [Fisher et al., 1997]. In many studies, UVA and UVB are studied independently [Bode and Dong, 2003]; however, for greater physiological relevance, it is recommendable to use the same ratio of UV wavelengths as that irradiated by the sun. The lamp we used emits UV consisting of 94.5% of UVA and 5.5% UVB. We irradiated 25 kJ/m^2 of solar UV for the HaCaT cells and human skin equivalent samples, corresponding to a single average UV daylight dose in New York City [Marionnet et al., 2015].

In this study, we observed that 20(*S*)-PPD has potential anti-wrinkle effects by suppressing solar UV-induced MMP-1 protein expression and MMP-1 transcription in HaCaT cells, to an extent greater than that achieved by Rb1 and GPPD. Solar UV is known to

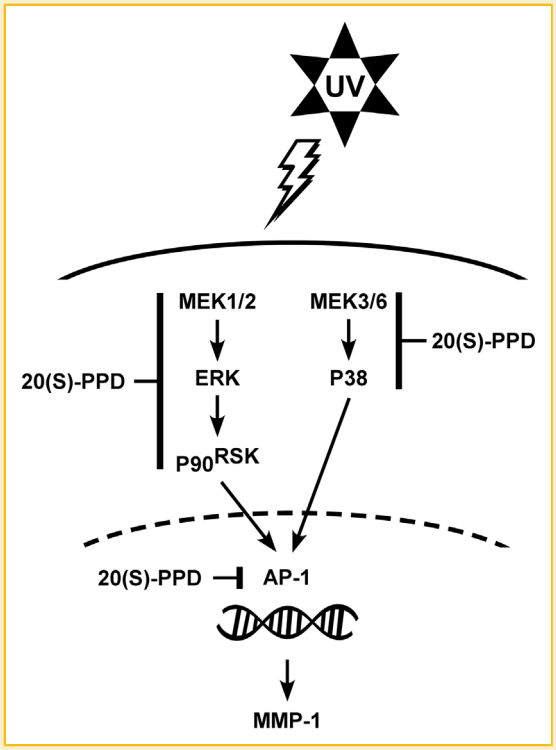

Fig. 6. Proposed mechanism of 20(*S*)-PPD. 20(*S*)-PPD inhibits solar UV-induced MMP-1 expression by regulating the MEK1/2/-ERK1/2-p90^{RSK} and MEK3/6-p38 pathways.

activate the MAPK and Akt pathways [Robinson and Cobb, 1997; Bode and Dong, 2003]. The components of AP-1 including c-jun and c-fos are in turn phosphorylated and activated through the MAPKs and Akt pathways [Whitmarsh and Davis, 1996]. By downregulating the phosphorylation of the MEK1/2-ERK1/2-p90^{RSK} and MEK3/6-p38 pathways, 20(*S*)-PPD inhibits AP-1 transactivation. However, we could not determine how 20(*S*)-PPD inhibits solar UV-induced MEK1/2-ERK-p90^{RSK} and MEK3/6-p38 pathways. Further research is ongoing to determine the mechanisms involved.

In conclusion, our findings indicate that 20(*S*)-PPD shows suppression activity against solar UV-induced MMP-1 expression in a human skin equivalent model and HaCaT cells. The effect of 20 (*S*)-PPD on MMP-1 transcription is regulated by decreased AP-1 activity via suppression of the MEK1/2-ERK1/2/p90^{RSK} and MEK3/6-p38 pathways (Fig. 6). 20(*S*)-PPD therefore shows potential for development as an ingredient in therapeutic anti-wrinkle products.

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