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Protective Effects of *Prunus persica* Flesh Extract (PPFE) on UV-Induced Oxidative Stress and Matrix Metalloproteinases Expression in Human Skin Cells

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Abstract – In our continuous efforts to procure the active materials from natural products in the protective effects of oxidative stress or UV damage to skin cells we found the *Prunus persica* flesh extract (PPFE) is considerable to meet the demand to protect the skin damage. PPFE attenuated cell damage induced by hypoxanthine-xanthine oxidase in cultured human keratinocytes, indicating that PPFE has the potential of the scavenging effect of reactive oxygen species (ROS) in human skin cell. Moreover, PPFE significantly suppressed UVA-induced ROS production determined by the oxidation of 2,7-dichlorodihydrofluorescein diacetate (DCFH) using FACS analysis. Additional study revealed that UVA irradiation of HaCaT human keratinocytes increased the gelatinolytic activities of matrix metalloproteinase-2, and -9 (MMP-2, -9) and mRNA expression of MMP-9 analyzing by a real-time reverse transcriptase-polymerase chain reaction (RT-PCR), and these events were significantly suppressed by the treatment with PPFE. These results suggest that PPFE might be applicable as natural ingredients for skin antiaging agents via UV-induced ROS scavenging activity and suppression of MMP expression in the skin cells.
Keywords – Matrix metalloproteinases, Oxidative stress, *Prunus persica* flesh extract, Skin aging, Ultraviolet A

Introduction

Since molecular mechanism of skin wrinkles seems to be due to the loss of collagen component of the dermal matrix by enhanced enzymatic degradation through the induction of matrix metalloproteinases (MMPs) (Gilchrest, 1996; West, 1994), the inhibition of MMPs appears to be useful intervention for skin-wrinkle (Fisher *et al*., 1999). The matrix metalloproteinases (MMPs), as known more than 24 human MMPs, are a family of zinc-binding endopeptidases capable of degrading extracellular matrix (ECM) components including collagen and proteoglycans (Nagase *et al*., 1999). The level of collagen in normal skin is maintained by the balance between synthesis by dermal fibroblasts and enzymatic degradation. However, in ultraviolet-irradiated skin, the level of MMPs, important enzymes for the proteolysis of ECM proteins, is elevated for long time before the visible symptoms of photoaging are appeared (Fisher *et al*., 1997). Indeed, among MMPs, gelatinases MMP-2 and MMP-9 are synthesized as proenzymes and play an important role in degrading type IV collagen (Berneburg *et al*., 1999; Johnson *et al*., 1998). Therefore, the inhibition of MMP induction has been reported to alleviate the UV-induced photoaging by preventing from collagen destruction (Kobayashi *et al*., 1998). Moreover, MMP-9 is known to be produced by a variety of cells including keratinocytes (Kahari *et al*., 1997), and also MMP-9 contributes to keratinocyte hyperproliferation in an oncogene-derived skin carcinogenesis (Coussens *et al*., 2000).

In addition, reactive oxygen species (ROS) production and oxidative processes associated with UV irradiation are considered as important components of photoaging. ROS also plays a role in the UV-induced expression of MMPs through redox regulatory transcription factors (Masaki *et al*., 1995). ROS includes free radicals such as the superoxide anion (O_2^-) and the hydroxyl radical ($OH\cdot$), as well as nonradical intermediates such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). There is evidence that UVA irradiation induces the generation of superoxide anion both in mitochondria and extramitochondrial sites (Gniadecki *et al*., 2000). The O_2^- is very toxic to tissue and may also result in the generation of ROS of other types. Hydrogen peroxide is

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formed by dismutation of superoxide anion in a spontaneous process that is enhanced by superoxide dismutase (SOD) (Petersen *et al*., 2000). Antioxidants play an important role that protects skin against ROS-induced injury. Numerous antioxidants, such as ascorbic acid (Dunham *et al*., 1982), α-tocopherol (Gensler *et al*., 1991), β-carotene (Mathews-Roth *et al*., 1987), selenium (Overvad *et al*., 1985), butylated hydroxytoluene (Black *et al*., 1978) and a mixture of dietary antioxidants (Black *et al*., 1975), have been reported to inhibit UV-induced skin carcinogenesis.

Although the biological activities of seeds (Fukuda *et al*., 2003) and flowers (Kim *et al*., 2002) of *Prunus persica* have been reported extensively, there is no documentation related to the fructus part of the extracts and examining whether PPFE possesses antioxidant activity and anti-UV-induced MMP expression. In the present study, we therefore investigated the potency of the inhibition on oxidative stress and UVA-induced MMP expression in human skin cells by PPFE.

Experimental

Plant Materials and Reagents – Persicae fruit was purchased from a peach supplier in Janghowon, Gyeonggi-Do, Korea. The samples were authenticated by Dr. N.S. Seong at the National Institute of Crop Science, RDA, Korea. All media for cell culture, fetal bovine serum (FBS), trypsin-EDTA solution (1X), and antibiotic-antimycotic solution, streptomycin, and penicillin were purchased from GIBCO-BRL (Grand Island, NY). Hypoxanthine, xanthine oxidase, MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were obtained from Sigma (St. Louis, MO). The fluorogenic probes 2',7',-dichlorodihydrofluorescein diacetate (DCFH-DA) and 2',7'-dichlorodihydro-fluorescein (DCF) were obtained from Cambridge Biosciences (Cambridge, UK). iQTM SYBR® Green Supermix was purchased from Bio-Rad Co. (Hercules, CA). All other chemicals used were of reagent grade.

Extraction – Peach (*Prunus persica*) fruits were purchased at the Gyeonggi Dong-Boo Fruit Agricultural Cooperative (Korea), where a voucher specimen (PE20060801) has been deposited. The fruits were washed with tap water, the pericarp and seed were removed, and the flesh was freeze-dried. The freeze-dried flesh of peach (100 g) was ground and extracted with 95% ethanol for 48 hours at room temperature. The ethanol extract (14.3 g) was then filtered, concentrated in a rotary vacuum evaporator. Stock solutions of PPFE were sterilized by filtration through 0.2 μm pore membrane and kept in the dark at –20 ºC until use.

Cell Culture – Human immortalized keratinocytes (HaCaT cells) were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in a humidified incubator at 37 ºC in a 5% CO_2 / 95% air atmosphere. Human skin fibroblast cells (ATCC CCL-110) were maintained in Minimum essential medium (MEM) with 1 mM sodium pyruvate, 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in a humidified incubator at 37 ºC in a 5% CO_2 / 95% air atmosphere.

Oxidative Stress by Hypoxanthine-Xanthine Oxidase – Keratinocytes cultured on tissue culture plates (6-well plates, Costar) pretreated with PPFE for 1 hour were exposed to hypoxanthine-xanthine oxidase system (HX–XO, 200 μM and 20 mU/mL, respectively) for 30 min. Cells were then washed twice with PBS, and fresh medium was added to the wells. The MTT assay was used for the assessment of cell viability.

UVA irradiation – Keratinocytes and skin fibroblasts were cultured on 3.5-cm culture dish (Nunclon, Roskilde, Denmark). The confluent cells starved with serum-free media for 24 hours were pretreated with various concentrations of PPFE for 24 hours. After a brief wash with PBS, cells were then incubated with PBS (1 mL). UVA irradiation was performed immediately as follows. Cells were irradiated under a Bio-Sun system illuminator (Vilber Lourmat, France) with a UV peak at 312 nm. Irradiation output was monitored by means of a Waldmann UV meter (Villingen-Schwenningen, Germany), and the dose of UVA irradiation was 320 mJ/cm^2. After UVA exposure, cells were added with fresh media in the presence of PPFE, and incubated for the indicated times for further analysis.

Cell Viability Assay – The viability of cells was measured using the MTT assay with minor modification. The assay is based on the conversion of the yellow MTT (3-[4,5-dimethylthiazol-2]-2,5 diphenyl tetrazolium bromide) to purple formazan crystals by mitochondrial succinate dehydrogenase in viable cells. Briefly, MTT (5 mg/mL in PBS) was used for the quantification of living metabolically active cells and added to keratinocytes damaged with HX-XO in 6-well plates at a final concentration of 0.5 mg/mL. Following a 4 hours incubation to allow its conversion into formazan crystals, the media were removed, and DMSO was added to dissolve formazan. Absorbance was read at 595 nm using a microplate reader

and the results were expressed as a relative percentage of vehicle-treated control incubations.

Flow Cytometric Analysis of DCF Oxidation by UVA-Induced Reactive Oxygen Species – The UVA-induced oxidative stress was measured with flow cytometric analysis using DCF oxidation in human keratinocyte cells. Briefly, the confluent HaCaT keratinocytes starved with serum-free DMEM were pretreated with various concentrations of PPFE for 24 hours. After a brief wash with PBS, cells were irradiated by UVA. Cells were washed with PBS and then loaded with DCFH-DA (30 μM) in DMEM media for 30 min. The cell pellets were resuspended in 1 mL of PBS and then analyzed immediately fluorescence signals of 10,000 cells with the FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ), and the distribution of histograms was calculated using CellQuest software (Becton-Dickinson, San José, CA).

Zymography – MMP-2 and MMP-9 enzymatic activities were determined by gelatin zymography. Human skin keratinocyte HaCaT (2.5×10^5 cells/mL) were seeded in 24-well plates and incubated for 48 hours. At 80% confluency, the cells were treated with PPFE (5 mg/mL) in serum-free media for 3 or 5 days. The culture supernatant was used for MMP-2 and MMP-9 enzymatic activities by gelatin zymography. All experiments, including zymography, were performed in the absence of serum. Protein samples were electrophoresed on a gelatin containing 10% SDS-polyacrylamide gel. After electrophoresis, the gel was washed twice with washing buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2.5% Triton X-100), followed by a brief rinsing in washing buffer without Triton X-100. The gel was incubated with incubation buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $CaCl_2$, 0.02% NaN_3, 1 μM $ZnCl_2$) at 37 °C. After incubation, the gel was stained with Coomassie Blue R-250 and proteolytic activities of MMPs were detected as clear bands with degradation of gelatin against a blue background.

Real-time PCR – Real-time PCR was employed to determine the gene expression of MMPs in human skin keratinocyte HaCaT cells. The analyses were performed as follows. Briefly, total RNA was extracted by the use of TRI reagent and reverse transcribed at 42 °C for 60 min in 20 μL of Reverse Transcription System (Promega, MI) with 0.5 μg of oligo$(dT)_{15}$ primer. Specific MMP primers were designed using Roche Applied System (Basel, Swiss) and custom synthesized by Bioneer Corporation (Seoul, Korea). The following sequences were used: MMP-2 F5'-ATAACCTGGATGCCGTCGT; MMP-2 R5'-AGGCACCCTTGAAGAAGTAGC-3'; MMP-9 F5'-GAACCAATCTCACCGACAGG-3'; MMP-9 R5'-GCCACCCGAGTGTAACCATA-3'; β-actin F5'-AGCACAATGAAGATCAAGAT-3'; β-actin R5'-TGTAACGCAACTAAGTCATA-3'. Real-time PCR was conducted on an Mini Opticon system (Bio-Rad, Hercules, CA), using 5 μL of reverse transcription product, iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA), and primers in a total volume of 20 μL. The standard thermal cycler conditions were employed: 95 °C for 20 s before the first cycle, 95 °C for 20 s, 56 °C for 20 s, and 72 °C for 30 s, repeated 40 times followed by 95 °C for 1 min, and 55 °C for 1 min. The threshold cycle (C_T), indicating the fractional cycle number at which the amount of amplified target gene reaches a fixed threshold from each well, was determined using by MJ Opticon Monitor software. Relative quantification, representing the change in gene expression from real-time quantitative PCR experiments between the PPFE group and untreated control group, was calculated by the comparative C_T method as published earlier (Livak *et al*., 1996). The data were analyzed using the equation $2^{-\Delta\Delta CT}$, where $\Delta\Delta C_T = [C_T$ of target gene $- C_T$ of housekeeping gene$]_{\text{treated group}} - [C_T$ of target gene $- C_T$ of housekeeping gene$]_{\text{untreated control group}}$. For the treated samples, evaluation of $2^{-\Delta\Delta CT}$ represents the fold change in gene expression, normalized to a housekeeping gene (β-actin) and relative to the untreated control.

Statistical Analysis – Data are expressed as means ± standard deviation (SD) for the indicated number of independently performed experiments. Student's *t*-test (SigmaStat 3.1, Systat software Inc.) was used for the determination of statistical significance. The difference was considered to be statistically significant when $P < 0.05$.

Results

Reactive oxygen species (ROS) play an important role in damage of skin keratinocytes. To investigate whether PPFE can protect skin keratinocyte injury induced by HX-XO, skin keratinocyte HaCaT cells were treated with various concentrations of xanthine oxidase (10 or 20 mU/mL), and the cytotoxicity induced by ROS was determined by MTT assay. As shown in Fig. 1, xanthine oxidase evoked the cytotoxicity of keratinocytes in a dose-dependent manner. The treatment of 10 or 20 mU/mL xanthine oxidase along with hypoxanthine (200 μM) exhibited the cell viability of 52 or 16%, respectively, but co-treatment of PPFE (10 mg/mL) in the same assay system significantly protected the cytotoxicity with 91 or

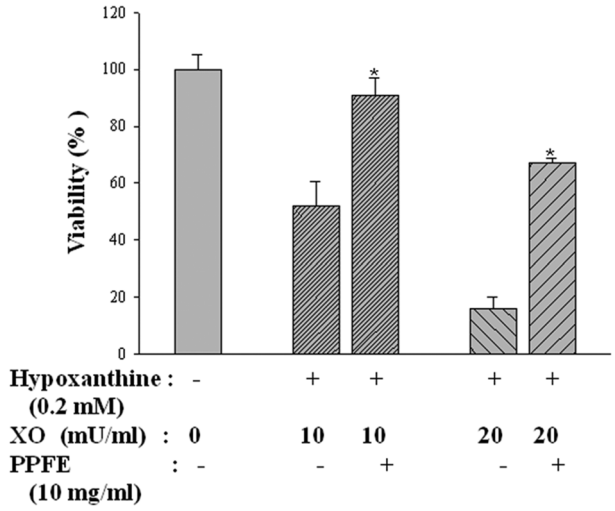

Fig. 1. Cytotoxicity evoked by hypoxanthine-xanthine oxidase system in HaCaT human keratinocytes. The cells were treated with xanthine oxidase as the indicated doses (10 or 20 mU/mL), and then cell viability was assessed by MTT assay as describe in Materials and Methods. Statistical analyses were performed using the Student's *t* test and one-way ANOVA. Data represent the means ± SD of four determinations. $*P < 0.05$ was considered statistically significant compared to control.

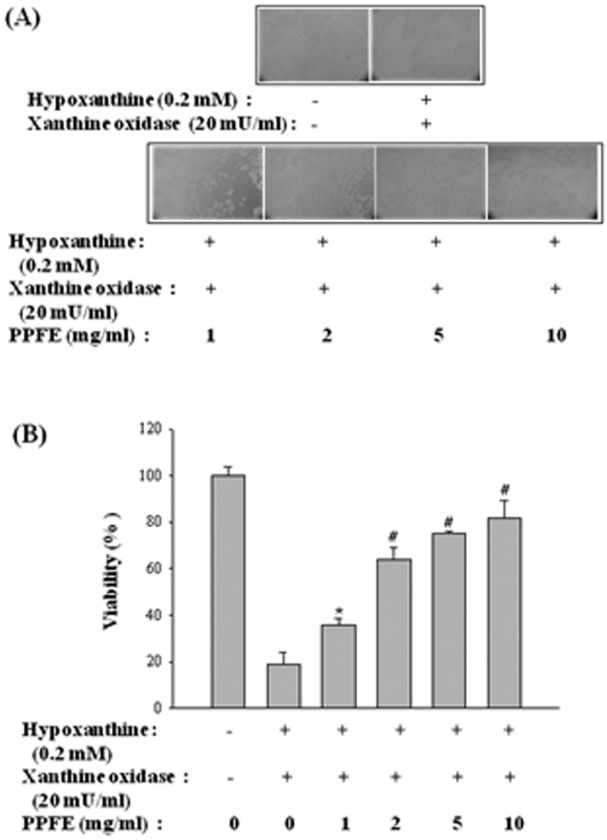

Fig. 2. Protective effects of PPFE on the skin cell death mediated by hypoxanthine-xanthine oxidase. (A) HaCaT cells treated with PBS (control) or hypoxanthine (HX)-xanthine oxidase (XO) (0.2 mM and 20 mU/mL) in the absence or presence of PPFE were analyzed under a phase-contrast microscope (Eclipse 4000, Nikon, Tokyo, Japan). The images were captured by a digital camera (Coolpix 950, Nikon) (x200) (B) HaCaT cells pretreated with various concentrations of PPFE for 1 hour were exposed to HX-XO (0.2 mM and 20 mU/mL) and incubated for an additional 1 hour. Cell viability was assessed by the MTT assay. The results are expressed as percentage of control and are means ± SD (n = 5). $*P < 0.01$, $^{\#}P < 0.05$ was considered statistically significant compared to control.

67% survival rate, respectively. This result suggested that PPFE might have a significant protective effect on HX-XO-induced skin keratinocyte cell damage.

The protective effect of PPFE on the inhibition of HX-XO-induced cell damage was also confirmed by the observation of cell morphology. As illustrated in Fig. 2A, the keratinocytes were distinctively damaged with HX-XO treatment compared to the normal control. However, the damage was alleviated by the treatment of PPFE. Racasan (Racasan *et al*., 2003) have demonstrated that HX-XO can induce ROS production in cells leading to cell death. We therefore sought to determine whether PPFE could protect HX-XO-induced cell death. The cytotoxicity in HX-XO-induced cell damage (200 μM hypoxanthine, 20 mU/mL xanthine oxidase) was 81% compared to control incubation. However, PPFE exhibited a dose-dependent protective effect on HX-XO-induced cell death as shown in Fig. 2B. Taken together, the HX-XO-induced cell damage was protected by PPFE treatment. These results suggest that PPFE might have a protective potential against ROS-induced cell damage.

Previous studies have shown that ROS is generated and is responsible for cell damage in cultured human skin cells during UVA irradiation (Tarozzi *et al*., 2005). We therefore evaluated whether PPFE can inhibit intracellular ROS production by UVA irradiation. The intracellular ROS produced in sin keratinocyte cells was measured using DCF-DA, a fluorescence dye for the detection of intracellular ROS, by flow cytometry (Katiyar *et al*., 2001). Flow cytometric analysis showed that mean fluorescence, i.e. intracellular ROS, was increased about 2.3 or 1.3-fold in skin keratinocyte cells (Fig. 3A) or skin fibroblast cells (Fig. 4A), respectively, by UVA-irradiated compared to unirradiated control cells. Treatment of PPFE (1, 2, or 5 mg/mL) dose-dependently inhibited the intracellular ROS production in UVA-irradiated skin keratinocyte cells (Fig. 3B). Moreover, the intracellular ROS production by UVA-irradiated skin fibroblast cells was strongly inhibited by PPFE (Fig. 4B). The observation indicates that PPFE might have a potential suppressive effect on the intracellular ROS production by UVA.

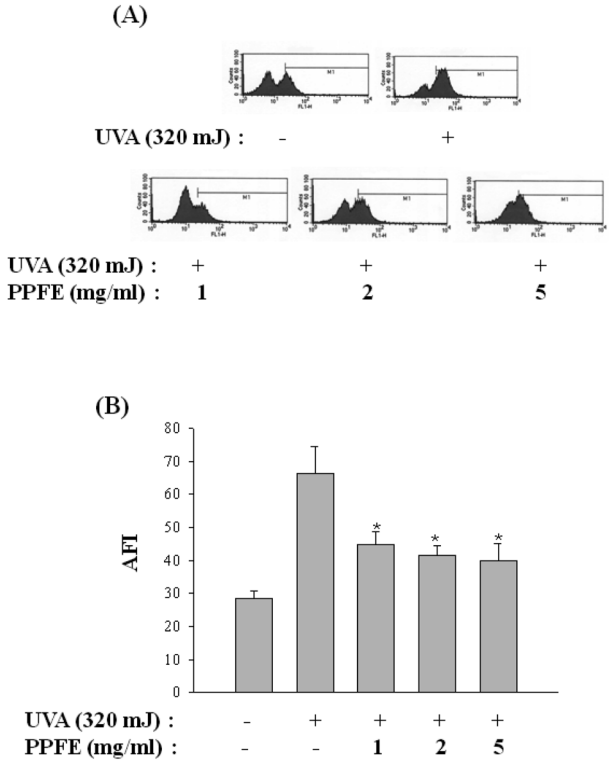

Fig. 3. Effect of PPFE on oxidative stress in UVA-irradiated (320 mJ/cm^2) HaCaT cells. **(A)** Flow cytometric analysis of DCFH oxidation. HaCaT cells preincubated with PBS or the indicated concentrations of PPFE were loaded with DCF-DA. After UVA irradiation, cells were collected and subjected to flow cytometric analysis. The results are presented as histograms of cell number versus fluorescence intensity. The data are representative of four independent experiments. **(B)** Quantitative analysis of DCF oxidation in HaCaT keratinocytes. The average fluorescence intensity (AFI) was determined by flow cytometric analysis. Results are shown as mean ± SD values from three independent experiments. *$P < 0.05$ was considered statistically significant compared to control.

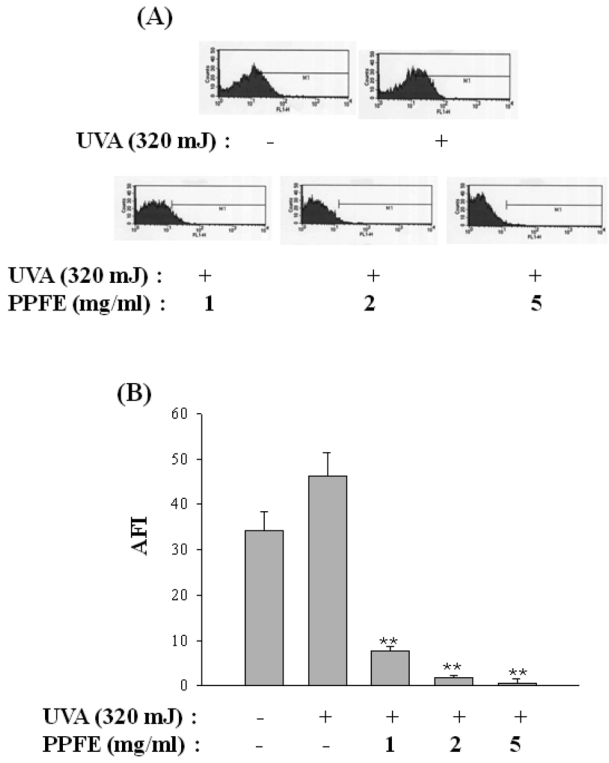

Fig. 4. Effect of PPFE on oxidative stress in UVA-irradiated (320 mJ/cm^2) human skin fibroblast cells. **(A)** Flow cytometric analysis of DCFH oxidation. Human skin fibroblast cells preincubated with PBS or the indicated concentrations of PPFE were loaded with DCF-DA. After UVA irradiation, cells were collected and subjected to flow cytometric analysis. The results are presented as histograms of cell number versus fluorescence intensity. The data are representative of four independent experiments. **(B)** Quantitative analysis of DCF oxidation in skin fibroblast cells. The average fluorescence intensity (AFI) was determined by flow cytometric analysis. Results are shown as mean ± SD values from three independent experiments. **$P < 0.01$ was considered statistically significant compared to control.

It is well known that matrix metalloproteinases (MMPs) are induced by the exposure of UVA and thus leading to the destruction of skin membrane. To investigate whether PPFE is able to suppress the expression of MMPs, human keratinocyte HaCaT cells were treated with PPFE (5 mg/mL) for 3 or 5 days, and the released MMP-2 and MMP-9 protein levels were determined by gelatin zymographic analysis. As shown in Fig. 5A, MMP-2 and MMP-9 proteins were constitutively expressed in untreated HaCaT cells and UVA (320 mJ/cm^2) exposure activated the expressions of MMP-2 and MMP-9. However, pretreatment with PPFE suppressed the UVA-induced activation of MMP-2 and MMP-9. Especially, the expression levels of activated MMP-2 and MMP-9 by UVA irradiation were significantly suppressed by the pretreatment of PPFE for 5 days (Fig. 5B and C). In addition, real-time PCR analysis showed that PPFE treatment also significantly suppressed the MMP-9 mRNA expression in UVA-irradiated HaCaT cells (Fig. 6).

Discussion

UVA irradiation (wave length; 320 - 400 nm) induces significant photodamaging effects, including carcinogenesis and aging due to the action of reactive oxygen species (Vile *et al*., 1995). Moreover, solar radiation induces the depletion of enzymatic and nonenzymatic antioxidant compounds in skin cells in situ, and this event leads to the enhancement of free radical reactions (Shindo *et al*., 1993).

Fruits and vegetables are considered as excellent functional foods because they contain high levels of

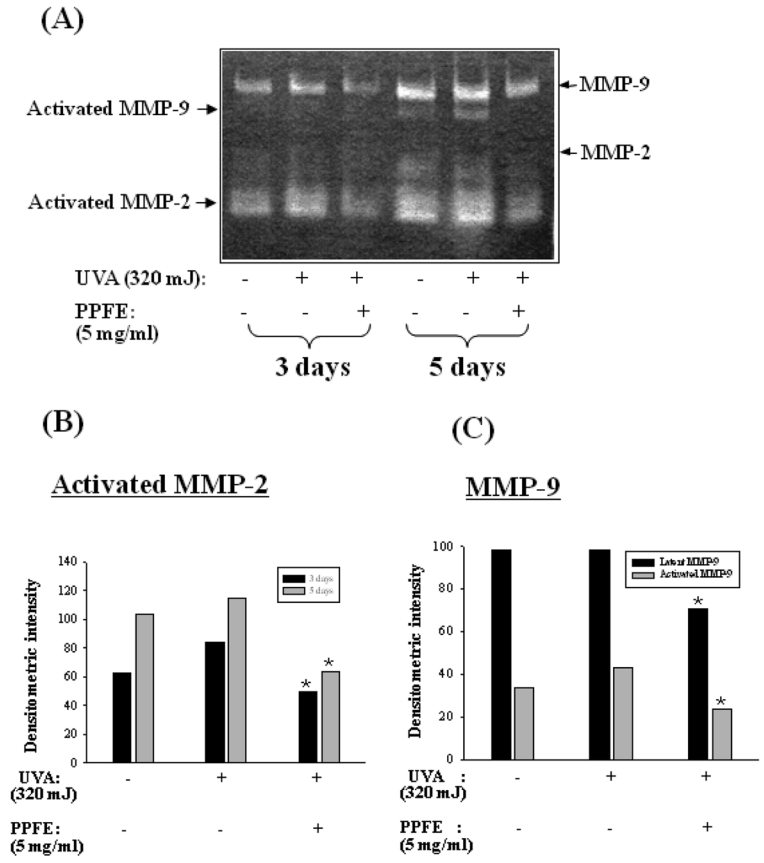

Fig. 5. Inhibitory effect of PPFE on UVA-induced MMPs activities. HaCaT cells pretreated with PPFE (5 mg/mL) were exposed to UVA irradiation (320 mJ/cm^2) and followed by incubation in medium in the absence or presence of PPFE. After 3 or 5 days, conditioned media were collected and analysed by gelatin zymography (A), followed by densitometric measurements of activated MMP-2 (B) and MMP-9 (C) after 5 days. Statistical analyses were performed using the Student's *t* test and one-way ANOVA. The data shown are the means ± SD of four determinations. *$P < 0.05$ was considered statistically significant compared to control.

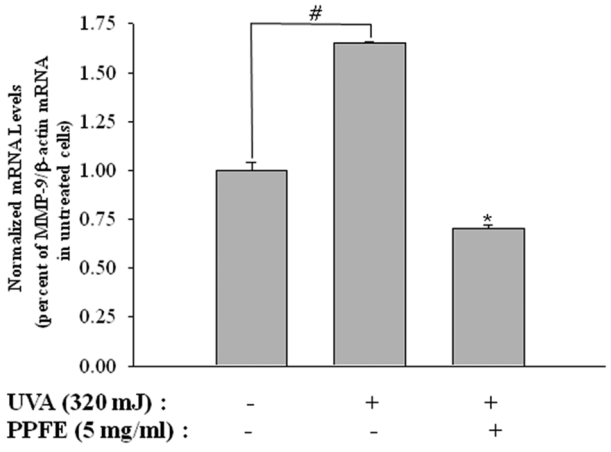

Fig. 6. Suppression of MMP-9 mRNA expression by PPFE in human HaCaT keratinocytes. HaCaT keratinocytes were treated with PPFE for 24 hours. The mRNA levels of MMP-9 were determined by real-time RT-PCR as described in Materials and Methods. Statistical analyses were performed using the Student's *t* test and one-way ANOVA. $^{\#,*}P < 0.05$ were considered statistically significant compared to control and control in UVA irradiation.

antioxidant compounds (Rupasinghe *et al*., 2007). Among them peach has health-promoting property by acting as antioxidants through scavenging harmful free radicals, which are implicated in most degenerative diseases and skin aging (Rice-Evans *et al*., 1996). Especially, our research group reported that PPFE attenuates chemotherapy-induced hepatotoxicity in mice and improves chemotherapeutic efficacy and protects against nephrotoxicity in cisplatin-treated (Lee *et al*., 2008a, 2009). In addition, we also reported the pericarp extract of *P. persica* attenuates chemotherapy-induced acute nephrotoxicity and hepatotoxicity in mice Lee *et al*., 2008b). Peach is also a nutritionally important source with one of the most important commodities consumed worldwide. Polyphenols are secondary plant metabolites, and they are the main sources of antioxidant capacity in peaches, although vitamin C and carotenoids also contribute to the capacity (Gil *et al*., 2002). The basic feature of all polyphenols is the presence of one or more hydroxylated aromatic rings,

which seemed to be responsible for their properties as radical scavengers (Fukumoto *et al*., 2000). Flavonoids are a large class of phenolic compounds, present in cereals, vegetables, and fruits, and these compounds are considered a significant contribution to the antioxidant capacity of fruits and vegetables (Prior *et al*., 2000). Anthocyanins are natural colorants with flavanols and flavonols, and are included in the flavonoid family. Anthocyanins have also potent antioxidant properties modulated by their different hydroxylations and glycosylations (Rice-Evans *et al*., 1996). The major anthocyanins in peach are reported with cyanidin-3-glucoside and cyanidin-3-rutinoside (Tomás-Barberán *et al*., 2001).

The antioxidative activities and inhibitory effects on the induction of MMPs are particularly focused since these properties appear to be major components of anti-photoaging actions. We report here that PPFE effectively blocked ROS induced by hypoxanthine-xanthine oxidase (HX-XO) system, and UVA irradiated production of ROS and the induction of MMPs (MMP-2 and -9) in human keratinocytes.

To elucidate the protective effect of PPFE against UVA-induced skin cell damage, primarily we determined whether PPFE is possible to alleviate the cell damage by ROS. ROS generated by exogenous HX-XO definitely induced skin keratinocyte cell death (Fig. 2), and PPFE protected the cell death in a concentration-dependent manner. This result showed that the protection of keratinocyte cell death might be related to the radical scavenging or antioxidant effects of PPFE.

The prolonged exposure of UVA to human skin is responsible for the higher levels of oxidative stress in skin cells, which leads to skin aging (Scharffetter-Kochanek *et al*., 1997). Indeed, hydrogen peroxide is generated in cultured human skin cells during UVA exposure (Fisher *et al*., 1999) and superoxide anion (O_2^-) is also generated in cells treated with HX-XO (Racasan *et al*., 2003). These reactive oxygen species appear to be responsible for the generation of strand breaks and alkali-labile sites in cultured human keratinocytes (Petersen *et al*., 2000). The results which PPFE inhibited UVA-induced ROS production in keratinocytes and skin fibroblasts (Figs. 3 and 4) and suppressed the cell damage induced by direct addition of HX-XO on keratinocytes (Fig. 2) indicated that PPFE specifically inhibits UVA-induced ROS production. This inhibition, therefore, leads to its protective effect against UVA-induced keratinocyte cell death. It is also known that ROS generated in UVA-irradiated human skin resulted in oxidative damage to lipids, proteins and DNA (Hanson *et al*., 2002). Especially, ROS produced by UV irradiation regulate gene expression including the induction of MMPs (Shang *et al*., 2002).

Since one of molecular mechanisms of skin wrinkles is highly related to the loss of collagen component of the dermal matrix by enhanced enzymatic degradation through the induction of MMPs (Gilchrest, 1996; West, 1994), the inhibition of MMPs seems to be useful for the intervention of skin wrinkle (Fisher *et al*., 1999). In addition, ROS production and oxidative processes by UV irradiation are considered as important components of photoaging, and the induction of the expression of MMPs through redox regulatory transcription factors (Masaki *et al*., 1995). In accordance with previous other reports, our data also demonstrated that UVA irradiation increased ROS production (Figs. 3, 4) and induced MMP-2 and -9 expressions, supporting the ROS involvement in UVA-induced MMPs expression. As shown in Fig. 5, however, the treatment of PPFE suppressed the UVA-induced enzyme activity and expression of MMP-2 and -9. More importantly, PPFE profoundly suppressed the ROS generation induced by UVA irradiation (Fig. 3, 4) and significantly reduced the mRNA expression of MMP-9 (Fig. 6). These results further imply that PPFE may have a protective potential against photoaging as well as ROS-mediated photodamage of the skin.

In conclusion, the present study provides the first evidence that PPFE inhibited UVA-induced ROS production and induction of MMP-2 and MMP-9 in HaCaT human keratinocytes. These results suggest that PPFE may act as an effective inhibitor of UVA-modulated signaling pathways and might be serving as an anti-photoaging agent.

Acknowledgments

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References

Berneburg, M., Grether-Beck, S., Kürten, V., Ruzicka, T., Briviba, K., Sies, H., and Krutmann, J., Singlet oxygen mediates the UVA-induced generation of the photoagingassociated mitochondrial common deletion. *J Biol Chem.* **274**, 15345-15349 (1999).

Black, H.S., Chan, J.T., and Brown, G.E., Effects of dietary constituents on ultraviolet light-mediated carcinogenesis. *Cancer Res.* **38**, 1384-

1387 (1978).
Black, H.S. and Chan, J.T., Suppression of ultraviolet light-induced tumor formation by dietary antioxidants. *J Invest Dermatol.* **65**, 412-414 (1975).
Coussens, L.M., Tinkle, C.L., Hanahan, D., and Werb, Z., MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell.* **103**, 481-490 (2000).
Dunham, W.B., Zuckerkandl, E., Reynolds, R., Willoughby, R., Marcuson, R., Barth, R., and Pauling, L., Effects of intake of Lascorbic acid on the incidence of dermal neoplasms induced in mice by ultraviolet light. *Proc Natl Acad Sci U S A.* **79**, 7532-7536 (1982).
Fisher, G.J., Talwar, H.S., Lin, J., and Voorhees, J.J., Molecular mechanisms of photoaging in human skin in vivo and their prevention by all-trans retinoic acid. *Photochem Photobiol.* **69,** 154-157 (1999).
Fisher, G.J., Wang, Z.Q., Datta, S.C., Varani, J., Kang, S., and Voorhees, J.J., Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med.* **337**, 1419-1428 (1997).
Fukuda, T., Ito, H., Mukainaka, T., Tokuda, H., Nishino, H., and Yoshida, T., Anti-tumor promoting effect of glycosides from *Prunus persica* seeds. *Biol Pharm Bull.* **26**, 271-273 (2003).
Fukumoto, L.R. and Mazza, G., Assessing antioxidant and prooxidant activities of phenolic compounds. *J Agric Food Chem.* **48**, 3597-3604 (2000).
Gensler, H.L. and Magdaleno, M., Topical vitamin E inhibition of immunosuppression and tumorigenesis induced by ultraviolet irradiation. *Nutr Cancer.* **15**, 97-106 (1991).
Gil, M.I., Tomas-Barberan, F.A., Hess-Pierce, B., and Kader, A.A., Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J Agric Food Chem.* **50**, 4976-4982 (2002).
Gilchrest, B.A., A review of skin aging and its medical therapy. *Br J Dermatol.***135**, 867-875 (1996).
Gniadecki, R., Thorn, T., Vicanova, J., Petersen, A., and Wulf, H.C., Role of mitochondria in ultraviolet-induced oxidative stress. *J Cell Biochem.* **80**, 216-222 (2000).
Hanson, K.M. and Clegg, R.M., Observation and quantification of ultraviolet-induced reactive oxygen species in ex vivo human skin. *Photochem Photobiol.* **76**, 57-63 (2002).
Johnson, L.L., Dyer, R., and Hupe, D.J., Matrix metalloproteinases. *Curr Opin Chem Biol.* **2**, 466-471 (1998).
Kahari, V.M. and Saarialho-Kere, U., Matrix metalloproteinases in skin. *Exp Dermatol.* **6**, 199-213 (1997).
Katiyar, S.K., Afaq, F., Azizuddin, K., and Mukhtar, H., Inhibition of UVB-induced oxidative stress-mediated phosphorylation of mitogen-activated protein kinase signaling pathways in cultured human epidermal keratinocytes by green tea polyphenol (−)-epigallocatechin-3-gallate. *Toxicol Appl Pharmacol.* **176**, 110-117 (2001).
Kim, Y.H., Yang, H.E., Park, B.K., Heo, M.Y., Jo, B.K., and Kim, H.P., The extract of the flowers of *Prunus persica*, a new cosmetic ingredient, protects against solar ultraviolet-induced skin damage in vivo. *J Cosmet Sci.* **53**, 27-34 (2002).
Kobayashi, T., Hattori, S., Nagai, Y., Tajima, S., and Nishikawa, T., Differential regulation of MMP-2 and MMP-9 gelatinases in cultured human keratinocytes. *Dermatology.* **197**, 1-5 (1998).
Lee, C.K., Park, K.K., Hwang, J.K., Lee, S.K., and Chung, W.Y., Extract of *Prunus persica* flesh (PPFE) improves chemotherapeutic efficacy and protects against nephrotoxicity in cisplatin-treated mice. *Phytother Res.* **23**, 999-1005 (2009).
Lee, C.K., Park, K.K., Hwang, J.K., Lee, S.K., and Chung, W.Y., The extract of *Prunus persica* flesh (PPFE) attenuates chemotherapy-induced hepatotoxicity in mice. *Phytother Res.* **22**, 223-227 (2008a).
Lee, C.K., Park, K.K., Hwang, J.K., Lee, S.K., and Chung, W.Y., The pericarp extract of *P. persica* attenuates chemotherapy-induced acute nephrotoxicity and hepatotoxicity in mice. *J Med Food.* **11**, 302-306 (2008b).
Livak, K.J. and Schmittgen, T.D., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). *Method.* **25**, 402-408 (1996).
Masaki, H., Atsumi, T., and Sakurai, H., Detection of hydrogen peroxide and hydroxyl radicals in murine skin fibroblasts under UVB irradiation. *Biochem Biophys Res Commun.* **206**, 474-479 (1995).
Mathews-Roth, M.M. and Krinsky, N.I., Carotenoids affect development of UVB induced skin cancer. *Photochem Photobiol.* **46**, 507-509 (1987).
Nagase, H. and Woessner, J.F. Jr., Matrix metalloproteinases. *J Biol Chem.* **274**, 21491-21494 (1999).
Overvad, K., Thorling, E.B., Bjerring, P., and Ebbesen, P., Selenium inhibits UV-light-induced skin carcinogenesis in hairless mice. *Cancer Lett.* **27**, 163-170 (1985).
Petersen, A.B., Gniadecki, R., Vicanova, J., Thorn, T., and Wulf, H.C., Hydrogen peroxide is responsible for UVA-induced DNA damage measured by alkaline comet assay in HaCaT keratinocytes. *J. Photochem Photobiol B.* **59**, 123-131 (2000).
Prior, R.L. and Cao, G.H., Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *Hort Science.* **35**, 588-592 (2000).
Racasan, S., Turkstra, E., Joles, J.A., Koomans, H.A., and Braam, B., Hypoxanthine plus xanthine oxidase causes profound natriuresis without affecting renal blood flow autoregulation. *Kidney Int.* **64**, 226-231 (2003).
Rice-Evans, C., Miller, N., and Paganga, G., Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med.* **20**, 933-956 (1996).
Rupasinghe, H.P.V. and Clegg, S., Total antioxidant capacity, total phenolic content, mineral elements, and histamine concentrations in wines of different fruit sources. *J Food Compos Anal.* **20**, 133-137 (2007).
Scharffetter-Kochanek, K., Wlaschek, M., Brenneisen, P., Schauen, M., Blaudschun, R., and Wenk, J., UV-induced reactive oxygen species in photocarcinogenesis and photoaging. *Biol Chem.* **378**, 1247-1257 (1997).
Shang, J., Schwarz, C., Sanchez Ruderisch, H., Hertting, T., Orfanos, C.E., and Tebbe, B., Effects of UVA and L-ascorbic acid on nuclear factor-kappa B in melanocytes and in HaCaT keratinocytes. *Skin Pharmacol Appl Skin Physiol.* **15**, 353-359 (2002).
Shindo, Y., Witt, E., and Packer, L., Antioxidant defense mechanisms in murine epidermis and dermis and their response to ultraviolet light. *J Invest Dermatol.* **100**, 260-265 (1993).
Tarozzi, A., Marchesi, A., Hrelia, S., Angeloni, C., Andrisano, V., Fiori, J., Cantelli-Forti, G., and Hrelia, P., Protective effects of cyanidin-3-O-beta-glucopyranoside against UVA-induced oxidative stress in human keratinocytes. *Photochem Photobiol.* **81**, 623-629 (2005).
Tomás-Barberán, F.A., Gil, M.I., Cremin, P., Waterhouse, A.L., Hess-Pierce, B., and Kader, A.A., HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches and plums. *J Agric Food Chem.* **49**, 4748-4760 (2001).
Vile, G.F. and Tyrrell, R.M., UVA radiation-induced oxidative damage to lipids and proteins in vitro and in human skin fibrobla-sts is dependent on iron and singlet oxygen. *Free Rad Biol Med.* **18**, 721-730 (1995).
West, M.D., The cellular and molecular biology of skin aging. *Arch Dermatol.* **130**, 87-95 (1994).

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