Inhibitory effects of *Prunus persica* flower extracts on UV-induced skin damage

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Abstracts

For an attempt to develop safe materials protecting UV-induced skin damage, plant extracts were evaluated for their antioxidative and free radical scavenging activities. From the results of these screening procedures, the ethanol extract of the flowers of *Prunus persica* was selected for further study. It was found that *Prunus persica* (50-200 ㎍/ml) inhibited UVB-induced DNA damage measured by tail moment in the Single Cell Gel Electrophoresis(COMET assay) and inhibited UV-induced lipid peroxidation, especially against UVB-induced peroxidation at higher than 10 ㎍/ml. Also *P. persica*(100~1,000㎍/㎖) inhibited the amount of ^{14}C-arachidonic acid metabolites release from UVB-irradiated keratinocytes and it possessed the protective activity against UV-induced cytotoxicity of keratinocytes. All these results indicate that the flowers of *P. persica* extract may be beneficial for protection UV-induced skin damage when topically applied.

Introduction

Besides of many beneficial effects including vitamin D metabolism and melanin synthesis, solar ultraviolet(UV) radiation also gives notorious effects leading to skin erythema, skin aging including premature wrinkle formation, and ultimately skin carcinogenesis[1,2]. They include activation of signal transduction pathways involving protein kinases and transcriptional factors[3], release of cytokines, formation of arachidonic acid(AA)/metabolites, oxidative stress and ultimately DNA damage. By UV irradiation, keratinocytes are activated and many cellular metabolisms are changed such as release of AA/metabolites from the membrane and formation of peroxidation products[4]. Amount of released AA by UV irradiation was found to be related with formation of prostaglandin E_2(PGE_2), one of chemical mediators of erythema, and closely associated with oxidative stress[5-7]. To prevent these harmful effects, several kinds of UV blocking agents/sunscreens have been currently used in cosmetic industry, which absorb or scatter UV light not to reach into epidermal and dermal layers.

However, there is a definite need of new materials to protect and/or prevent UV-induced photo-damage for safer and long-term uses. One example is an application of anti-oxidants such as vitamin C and/or E, which reduce oxidative damege caused by UV irradiation[8-10], although there are always problems of stabilization of the final formulations. Other possible approach may be finding safe plant extracts to protect metabolic alterations of skin cells, especially epidermal layer, one of the sites responsible for photo-damaging reactions. Also, it is reasonable to think that materials protecting metabolic alteration of keratinocytes by UV exposure could have a beneficial effect on skin via preventing photo-damage. For an attempt to develop plant materials protecting UV-induced skin damage, we have checked inhibitory potential of 200 plant extracts against free radical formation. From the results of these screening procedures, the ethanol extract of the flowers of *Prunus persica* was selected for further study. This paper deals with the protective effects of *Prunus persica* against AA/metabolites release of human keratinocyte culture and DNA damgae in fibroblast.

Material & Methods

Plant materials : The flowers of *Prunus persica* (Rosaceae) were collected in several orchards located in Kangwon province (Korea) in April (1997 and 1998) and dried in the dark. The plant material (1Kg) was extracted in 80% aqueous ethanol (10L) at room temperature for 7 days. The extract was filtered and dried under vacuo giving 172g of crude extract. For cell culture study, the plant extracts were dissolved in PBS.

Single cell gel electrophoresis. Cells were embedded in agarose on frosted microscope slides by the method of Sing et al[11]. First, 0.65% NMPA in PBS (100 μ l) at 65° C was dropped onto slides and they were covered with glass coverslip (18 x 18mm, No.1). After leaving on ice for 10 min, the cover slip was removed. Cells were mixed with 200 μ l of 0.5% LMPA and 50 μ l cell suspension was immediately pippeted onto the layer of agarose on the same slide. After covering with a coverslip, the slide was left on ice for 10 min. A final layer of agarose (100 μ l of 0.5% LMPA) was applied in the same way. The slide without coverslip was immersed in ice-cold lysis solution (10 mM Tris, pH 10.0, containing 2.5 M NaCl, 100 mM EDTA, 10% DMSO and 1% Triton X-100) at 4° C for 1 hr. Electrophoresis was carried out in a tank containing 300 mM NaOH, 1 mM EDTA, pH 13.0 for 15 min under 25 V and 300 mA. Slides were then transferred to 0.4 M Tris buffer (pH 7.5), washed three times and gently dried. Ethidium bromide (2 μ g/ml) was dropped onto the gel to stain DNA. Slides were examined at X400 magnification using a BH2 fluorescence microscope (Olympus, Japan) equipped with a 20BG-W2 dichroic mirror (excitation filter: 515 nm, barrier filter: 590 nm). Image analysis was performed

with the software Komet (version 3.0, Kinetic Imaging, Livepool, UK) on 25 randomly selected cells. DNA damage was quantified by the increase of the tail moment, which was defined as a product of comet length and amount of DNA in the tail [12]

Arachidonic acid (AA) labeling and release study : Keratinocytes were plated in 24-well plates (2×10^4 cells/well). When culture was 80-90% confluent, cells were labeled with 0.0375 μ Ci/㎖ [^{14}C]AA in KGM without supplements for 24 hrs. The media was removed and washed 3 times with KGM containing 1 mg/ml fatty acid free- BSA. For checking distribution pattern of ^{14}C-AA in the membrane, cells were extracted with chloroform : methanol (2:1) and organic phase was dried under N_2. Lipids were analyzed using TLC with chloroform : methanol : acetic acid : water (90:8:1:0.8) as a mobile phase, in which glycerophospholipids remain on original spotting area. For examining the inhibitory effects of the plant extracts, cells were pre-incubated with extracts for 1hr. Immediately before irradiation, the medium was replaced by small amount of calcium/magnesium-free PBS to avoid drying during UVB irradiation. The PBS contained the extract in the same concentration as in the pre-incubation, while control group received same amount of PBS. After UVB (30mJ/㎠) was irradiated, KGM containing the same amount of the extract was re-added and cells were incubated further for 6hrs. The medium was removed and washed with KGM containing 1mg/ml fatty acid-free BSA twice. The combined medium was centrifuged to remove cell debris. Radioactivity of small amount of the supernatant was measured in toluene-based scintillant and considered as total release (^{14}C-AA/^{14}C containing other cellular metabolites). For measuring ^{14}C-AA release, the remaining supernatant was extracted with chloroform : methanol (2:1). The organic layer was dried under N_2 and ^{14}C-AA was separated with TLC using petroleum ether : diethyl ether : acetic acid (50:50:1) as a mobile phase according to the procedure of Kim et al [13]. ^{14}C-AA released co-migrating with authentic standard was scraped out after visualization with iodine vapor and radioactivity was counted. Sometimes, autoradiography for 7 days was carried out to find radioactive spots on TLC plates.

Results and Discussion

UV can produce the deleterious changes of DNA structure directly or by peroxidation products generated. The damaged DNA, if not repaired correctly, may cause gene mutation and skin carcinogensis. For finding the materials to prevent these processes, the antioxidative potential and free radical scavenging activity were measured using about 200 plant extracts. From these screening procedures, *Prunus persica* extracts(Peach

flower extracts) was found to be one of the promising materials for protecting UV-induced DNA damage, and studied further in this investigation using the COMET assay.

Fig. 1 represented the dose-response curves of NIH/3T3 fibroblasts exposed to UVB in the COMET assay. From these results, UVB (50 mJ/cm^2) was used to evaluate the inhibitory effects of plant extracts against UV-induced DNA damage. *P. persica* extracts showed a significant reduction of the tail moment in the COMET assay (Fig. 2). The IC_{50} values of *P. persica* were found to be approximately 162 ug/ml in UVB-induced DNA damage. These findings strongly suggest that *P. persica* may protect DNA damage by UV irradiation. In contrast, dl-α -tocopherol used as a reference compound showed a reduction of the tail moment at low concentration while the fact that DNA damage was increasing at high concentrations of tocopherol might reflect its pro-oxidant property as previously described by Mak *et al* [14].

The cellular mechanism of *P. persica* exerting inhibition against UVB- induced DNA damage in fibroblasts is not clear at present. Most of the cellular DNA damage is caused by UV wavelength around 300 nm [15,16]. UV-absorption maximum of *P. persica* in methanol was found to be 330 and 298 nm (data not shown). This suggests that the inhibitory effect of *P. persica* may be partly due to sunscreen effect of UVB. In addition, since single cell gel electrophoresis (SCGE) is a powerful technique for the detection of DNA breaks in many eukaryotic cells, it is thought that *P. persica* may prevent DNA strand breaks induced by ROS generated. This speculation may be partly supported by the findings that *P. persica* inhibited lipid peroxidation induced by UV irradiation dose-dependently (Fig. 3), especially UVB-induced lipid peroxidation at higher than 10 μg/ml. However, the other possible mechanisms such as a modulation of DNA repair activity could not be excluded.

Epidermis is one of the sites for this change, in which keratinocytes are major cells. Thus, keratinocyte activation by UV irradiation is an important factor for skin erythema, premature aging and skin carcinogenesis. For the materials to prevent this process, the effects on AA/metabolites release from UVB-activated keratinocytes were measured, in addition to their inhibitory activity of free radical formation. From these screening procedures, the flowers of *P. persica* extract was found to be one of the promising materials for protecting UV-induced skin damage and studied further in this investigation.

Under the standard condition, *P. persica* extracts were added and UVB was irradiated. The radioactivity released to medium for 6hrs period was counted and considered as total release (^{14}C-AA /^{14}C -containing other cellular metabolites). UVB ($30mJ/cm^2$) irradiation increased total release from keratinocytes about 1.7-2.5 fold compared to the amount of sham irradiated group depending on the experiments. When *P. persica* was added and ^{14}C-

AA releasd was analyzed using TLC separation and autoradiographic techniques, the amounts of ^{14}C-AA as well as total radioactive materials released were significantly reduced in a concentration-dependent manner at 100 – 1,000 ㎍/㎖. The IC_{50} values of *P. persica* were found to be approximately 429 and 207 ㎍/㎖ for ^{14}C-AA and total release, respectively(Fig. 4).

When protective activity of *P. persica* against UVB-induced cytotoxicity was measured using MTT bioassay, UVB irradiation (30mJ/ cm^2) decreased viability of keratinocytes (31 ± 4%) while *Prunus persica* (100 – 1000 ㎍/㎖) significantly reversed UVB-induced cytotoxicity of keratinocytes (Data not shown). These findings strongly suggest that *P. persica* may protect keratinocyte activation and epidermal damage by UVB irradiation(Fig.5).

All results from this investigation indicate that *Prunus persica* clearly possesses protective activity against UV-induced skin damage judged by inhibitions of AA/metabolites release, cytotoxicity of keratinocytes and skin fibroblasts The investigation to elucidate the detailed action mechanism of UV protection is now under progress.

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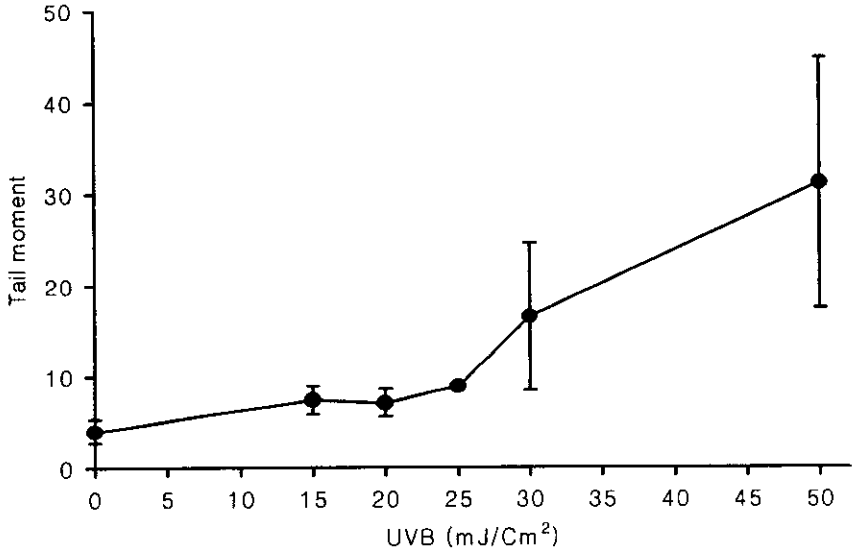

Fig. 1. UVB-induced DNA damage of NIH/3T3 cells in the COMET assay

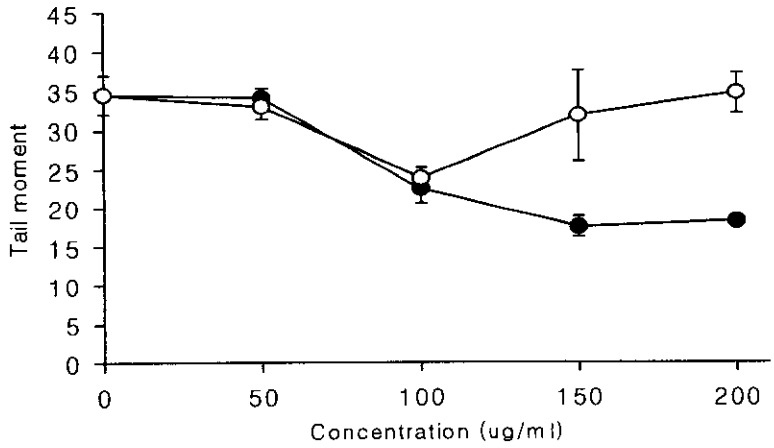

Fig. 2. Protective effects of the flowers of *Prunus persica* extract against UVB-induced DNA damage in the COMET assay, Tocopherol (○), P.persica extracts (●).

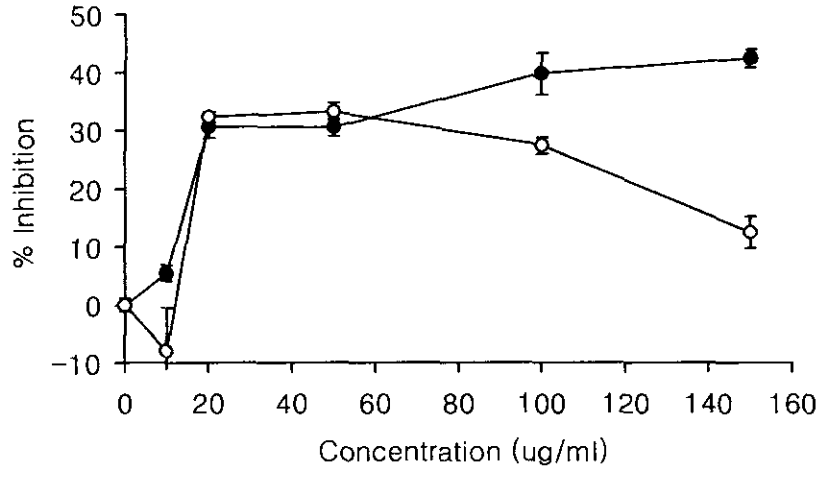

Fig. 3. Inhibition of UVB-induced lipid peroxidation by the flowers of *Prunus persica* extract Tocopherol (0), P. persica extracts (●).

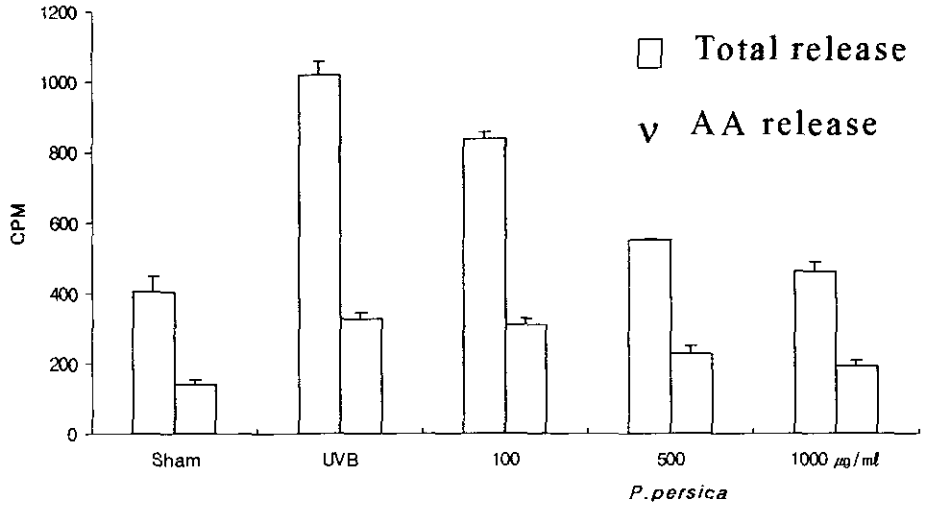

Fig.4. Concentration-dependent effects of the flowers of *P.persica* extract on ^{14}C-AA release from UVB irradiated keratinocytes

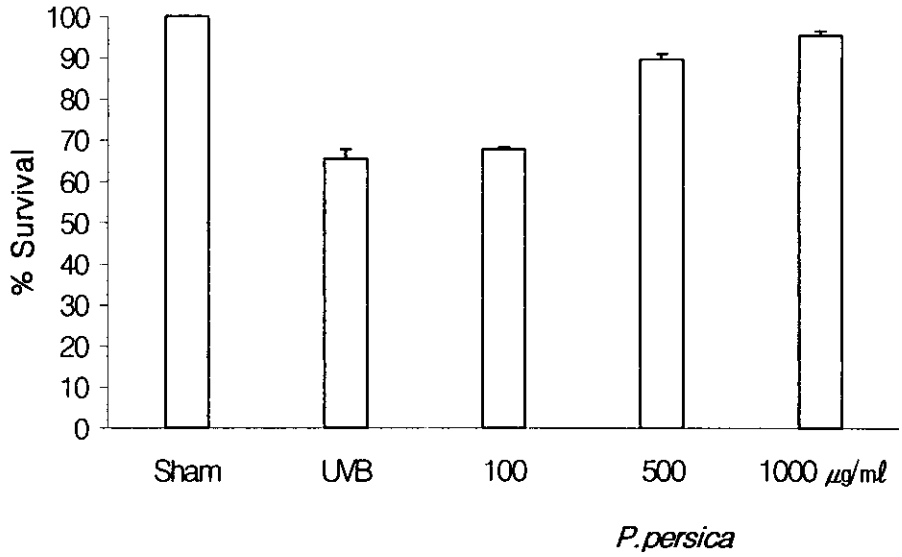

Fig. 5. Concentration-dependent effects of the flowers of *P. persica* extract on cytotoxicity of UVB irradiated keratinocytes.