JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

*J. Agric. Food Chem.* **2008,** *56,* 8969–8974 **8969**

# Anthocyanins from Black Soybean Seed Coats Inhibit UVB-Induced Inflammatory Cylooxygenase-2 Gene Expression and $PGE_2$ Production through Regulation of the Nuclear Factor-$\kappa$B and Phosphatidylinositol 3-Kinase/Akt Pathway

KONSTANTIN TSOYI,[†,‡] HYEONG BIN PARK,[‡,§] YOUNG MIN KIM,[†] JONG IL CHUNG,[||] SUNG CHUL SHIN,[⊥] WON SUK LEE,[#] HAN GEUK SEO,[†] JAE HEUN LEE,[†] KI CHURL CHANG,*[,†] AND HYE JUNG KIM*[,†]

Department of Pharmacology, School of Medicine and Institute of Health Sciences, Department of Orthopedic Surgery, School of Medicine, Department of Agronomy, Research Institute of Life Science, and Department of Chemistry, Research Institute of Life Science, Gyeongsang National University, and Department of Pharmacology, School of Medicine, Busan National University

Ultraviolet (UV) radiation can cause inflammatory changes and may further contribute to skin carcinogenesis. Anthocyanins are known to be powerful antioxidants that help protect plants from UV damage. Recently, we isolated anthocyanins from black soybean [*Glycine max* (L.) Merr] seed coats. Thus, we investigated the protective effect of anthocyanins from black soybean seed coats on UVB radiation-induced inflammatory responses and the molecular mechanism responsible for regulation of apoptosis and inflammatory responses. Anthocyanins inhibited UVB-induced cylooxygenase-2 (COX-2) and $PGE_2$ production through a nuclear factor-$\kappa$B-dependent pathway and regulation of the PI3 kinase/Akt pathway activated by UVB in a human keratinocyte cell line, HaCaT. Topical application of anthocyanins prior to UVB irradiation of hairless mice also inhibited induction of COX-2 and $PGE_2$. In conclusion, it is suggested that anthocyanins from the seed coat of black soybeans can be used as a useful drug to modulate oxidative disorders including UVB-induced inflammation.

**KEYWORDS: Anthocyanins; COX-2; NF-$\kappa$B; PI3 kinase/Akt; $PGE_2$; UVB**

## INTRODUCTION

Ultraviolet (UV) irradiation accounts for most of the harmful biological effects associated with sunlight, including skin aging and malignant transformations in mammals (*1, 2*). In particular, UVB has been shown to damage biological macromolecules, including lipids, proteins, and nucleic acids. UVB plays a major role in the development of human skin cancer (*3, 4*) and has been shown to act as a tumor initiator and tumor promoter in animals (*5, 6*). The inflammatory changes in response to acute UVB exposure of the skin include erythema, the production of inflammatory mediators, alteration of vascular responses, and inflammatory cell infiltrate. Alterations in cutaneous and systemic immunity occur as a result of UV-induced inflammation and damage, including changes in the production of cytokines by keratinocytes and other skin-associated cells, alteration of adhesion molecule expression, and loss of antigen-presenting cell function within the skin (*7*). These events impair the capacity of the immune system to eliminate cancerogenic-altered cells. Hence, the observed inflammatory response following acute and chronic UVB exposures may further contribute to skin carcinogenesis by oxidative stress mechanisms. UV radiation-induced lipid peroxidation increases the production of prostaglandins (PGs) including $PGE_2$, which in turn causes inflammation in the skin (*8*). $PGE_2$ is produced from arachidonic acid by the inducible form of cyclooxygenase, COX-2 (cylooxygenase-2). Numerous studies have shown that UVB irradiation significantly increases COX-2 gene expression at both mRNA and protein levels in human skin as well as cultured keratinocytes (*9*−*12*). The resulting synthesis of PGs might contribute to UV radiation-induced skin carcinogenesis (*13*). Because of its specific upregulation in the epidermis during a chronic exposure to UVB radiation, the COX-2 level may

* To whom correspondence should be addressed. (K.C.C.) Tel: +82-55-751-8771. Fax: +82-55-759-0609. E-mail: kcchang@gsnu.ac.kr. (H.J.K.) Tel: +82-55-751-8774. Fax: +82-55-759-0609. E-mail: hyejungkim@gnu.ac.kr.

† Department of Pharmacology, Gyeongsang National University.

‡ Contributed equally to this work.

§ Department of Orthopedic Surgery, Gyeongsang National University.

|| Department of Agronomy, Gyeongsang National University.

⊥ Department of Chemistry, Gyeongsang National University.

# Department of Pharmacology, Busan National University.

10.1021/jf801345c CCC: $40.75 
Published on Web 09/09/2008

actually be used as an early marker for potential skin tumorigenesis (*9, 11*). Convincing evidence exists to show that oral administration of celecoxib, a selective inhibitor of COX-2, significantly decreases the incidence and number of mouse skin cancers induced by solar-simulated radiation (*10*). Taken together, these results show not only the role of arachidonic acid/PGs pathway in photocarcinogenesis but also the therapeutic value of COX-2 inhibitors in the prevention of UV radiation-induced skin cancers.

The most convincing approach to confirm the involvement of oxidative stress in carcinogenesis might arise from studies in which antioxidants have been shown to effectively modulate photocarcinogenesis. A number of biologically active molecules with antioxidant properties have been shown to reduce UV carcinogenesis in mice. Topical application of (−)-epigallocatechin-3-gallate (EGCG), the major polyphenolic constituent in green tea, inhibited UVB-induced infiltration of leukocytes, reduced myeloperoxidase activity, and decreased UVB-induced erythema in human skin (*14, 15*). In several mouse skin models, treatment with green tea phenols has been shown to afford protection against chemical- as well as UVB-induced skin carcinogenesis and inflammatory responses (*16, 17*). However, most of these compounds have not been adequately tested for human effectiveness and safety in clinical trials. Therefore, there is a need for another strategy to support both safety and efficacy in humans.

With regards to the safety and efficacy mentioned above, anthocyanins from fruits such as berries and grapes are very promising. Anthocyanins act as powerful antioxidants that help to protect the plant from UV damage. Anthocyanins are abundantly present in chokeberry, cherry, aubergine, blue grape, Vaccinium and red cabbage, as well as the Usambara-violet. In addition, it is known that anthocyanins are especially abundant in the epidermis palisade layer of the black soybean seed coat (*18*−*23*). Three main anthocyanins, that is, cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside, were characterized in black soybean seed coats (*19, 21, 23*). Anthocyanins have been shown to have high antioxidant activity, which may be partially responsible for their anticarcinogenic activity (*24*).

Our previous study reported that anthocyanins from black soybean seed coats reduced ischemic reperfusion (I/R)-induced myocardial injury in vivo and inflammatory gene expression such as COX-2, which is known to be responsible for I/R injury (*25*). Because anthocyanins act as powerful antioxidants helping to protect the plant from UV damage, we hypothesized that anthocyanins from black soybean seed coats might inhibit UV-induced inflammatory responses. Thus, in this study, we aimed to investigate the protective effect of anthocyanins from black soybean [*Glycine max* (L.) Merr] on UVB-induced inflammatory responses and investigate the molecular mechanism by which anthocyanins regulate inflammatory responses.

## MATERIALS AND METHODS

**Materials and Chemicals.** Anthocyanins, extracted from black soybean [*G. max* (L.) Merr] and purified as described by Kim et al. (*25*), were provided by Dr. Sung Chul Shin (Department of Chemistry, Research Institute of Life Science, Gyeongsang National University). The compositions of anthocyanin were consisted of cyanidin-3-glucoside (72%), delphinidin-3-glucoside (20%), and petunidin-3-glucoside (6%). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and antibiotics (penicillin/streptomycin) were obtained from Gibco BRL (Rockville, MD). The anti-p-extracellular regulated kinase (ERK)1/2, anti-t-ERK1/2, anto-p-p38, anti-t-p38, anti-p-c-Jun N-terminal kinase (JNK), anti-t-JNK, anti-p-Akt, and anti-t-Akt antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals including anti-COX-2 antibody were supplied by Sigma (St. Louis, MO).

**Animals.** Eight to 10 week old adult female hairless mice (HR-1) were provided by Japan SLC Inc. (Shizuoka, Japan). All experiments were performed in compliance with institutional guidelines set by the Institutional Animal Care and Use Committee at the Gyeongsang National University.

**Cell Culture.** The immortalized human keratinocyte cell line, HaCaT, was kindly provided by Professor N. Fusenig (German Cancer Research, Germany). The cells were maintained as monolayer cultures in DMEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 $\mu$g/mL streptomycin at 37 °C in a humidified atmosphere containing 5% $CO_2$. The cells were seeded at a density of $1 \times 10^6$ cells/100 mm dishes. After 48 h, the cells were starved with serum-free medium for 12 h prior to anthocyanins treatment. Cells were used for experiments until passage number 20.

**UV Irradiation to HaCaT.** UVB irradiation was performed on serum-starved monolayer cultures utilizing a FS20 Lamp (National Biological, Twinsburg, OH). The UVB source was a bank of two FS20 Lamps emitting a continuous spectrum from 270 to 390 nm, with a peak emission at 313 nm; approximately 65% of the radiation was within the UVB (280−320 nm) wavelength. The irradiance of the single bulb averaged 6.25 J/m$^2$/s at a tube-to-target distance of 8 cm, and the irradiance of the bank of two bulbs averaged 12 J/m$^2$/s, as measured by a spectroradiometer (model IL1700A; International Light, Inc., Newburyport, MA). The cells were irradiated with UVB in a minimum amount of PBS (about 3 mL). Control cells were sham irradiated. After UVB irradiation, the cells were cultured in serum-free medium for the indicated time with or without anthocyanins or other drugs.

**UVB Irradiation of Animals and Preparation of Skin Samples.** Fifteen hairless mice were divided into five groups of three animals each and exposed to UVB irradiation (2.4 kJ/m$^2$). UVB irradiation to animals was performed as described by Kim et al. (*26*). Immediately after UVB irradiation, anthocyanins (at a dose of 50 or 100 mg/kg in sterile water, 100 $\mu$L) were applied to the mice. Twenty-four hours later, mice were anesthetized (pentobarbital sodium, 50 mg/kg) and killed by exsanguinations from the retro-orbital plexus. The dorsal skin (ca. 1 × 2 cm) excised from each mouse was placed on ice and immediately frozen at −80 °C until it was used for Western blotting and $PGE_2$ ELISA assay.

**Western Blot Analysis.** Total cell lysates were obtained using lysis buffer containing 0.5% SDS, 1% NP-40, 1% sodium deoxycholate, 150 mM NaCl, 50 mM Tris-Cl (pH 7.5), and protease inhibitors. The protein concentration of each sample was determined using a BCA protein assay kit (Pierce, Rockford, IL). To detect COX-2, 20 $\mu$g of total protein was electrophoresed on an 8% polyacrylamide gel, and to detect phospho-mitogen-activated protein kinases (MAPKs), phosphor-Akt, or phosphor-I$\kappa$B$\alpha$, 30 $\mu$g of total protein was electrophoresed on a 12% polyacrylamide gel. To detect nuclear factor-$\kappa$B (NF-$\kappa$B) (p65), nuclear and cytosolic fractions were separated as described by Nizamutdinova et al. (*27*), and 30 $\mu$g aliquots of isolated nuclear or cytosolic protein were subjected to 10% SDS-polyacylamide gel. The separated proteins were transferred to polyvinylidene fluoride membrane (Amersham) for 2 h at 20 mA with a SD Semidry Transfer Cell (Bio-Rad). The membranes were blocked with 5% nonfat milk in Tris-buffed saline (TBS) containing 0.05% Tween 20 (TBS-T) for 2 h at room temperature. The membranes were then incubated with primary antibodies at a concentration of 1:500 in 5% skim milk TBS-T overnight at 4 °C. Bound antibody was detected by horseradish peroxidase-conjugated secondary antibodies, and signals were detected by an enhanced chemoluminescence (ECL) method (Amersham, Piscataway, NJ).

**Transfection.** Construction of the specific regions (−1917/+127) of the human COX-2 gene promoter in the luciferase reported pGL3 Basic (Promega, Madison, WI) was accomplished by polymerase chain reaction (PCR) amplification. NF-$\kappa$B-luciferase constructs (consensus NF-$\kappa$B binding sequence was cloned into the pGL3 basic luciferase expression vector) were kindly provided by Dr. G. Koretzky (University of Pennsylvania), and the dominant negative I$\kappa$B mutant plasmid (pcDNA/I$\kappa$B$\alpha$-SR) was a gift from Dr. H. Nakshatri (Indiana University School of Medicine, Indianapolis, IN). Transient transfections were

performed using Lipofectin (Gibco-BRL) according to the manufacturer's protocol. Briefly, $5 \times 10^5$ cells were plated in a 60 mm dish the day before transfection and grown to about 70% confluence. Two micrograms per plate of plasmid DNA prepared by Qiagen kit (Qiagen, Santa Clara, CA) were transfected. Transfections were allowed to proceed for 12 h. The transfected cells were washed with 4 mL of PBS, treated with anthocyanins for 24 h, and then irradiated with UVB. The cells were continually cultured in serum-free DMEM until they were harvested. The transfection efficiency was corrected by cotransfection of 0.5 $\mu$g of phRL-TK Vector (Promega).

**Luciferase Assay.** After experimental treatments, cells were washed twice with cold PBS, lysed in a passive lysis buffer provided in the dual luciferase kit (Promega, Madison, WI), and assayed for luciferase activity using the TD-20/20 luminometer (Turner Designs, Sunnyvale, CA) according to the manufacturer's protocol. All transfections were performed in triplicate. Data are presented as a ratio between Firefly and Renilla luciferase activity.

**Quantitative Human $PGE_2$ Immunoassay.** The quantity of $PGE_2$ secreted into the culture medium was analyzed from culture media using a Quantikine human $PGE_2$ chemiluminescence kit (R&D Systems), according to the manufacturer's manual. To quantify the $PGE_2$ from mouse skin, 1 mL of extraction buffer (0.1 mol/L phosphate, pH 7.4, containing 1 mmol/L EDTA, and 10 $\mu$mol/L indomethacin) was added to 1 g of tissue sample and homogenated. The supernatant was used for analysis. Both the samples and standards were assayed in parallel.

**Data Analysis.** Scanning densitometry was performed using an Image Master VDS (Pharmacia Biotech Inc., San Francisco, CA). Treatment groups were compared using one-way analysis of variance (ANOVA), and the Newman−Keuls test was used to locate any significant differences identified in the ANOVA. The data are presented as the means ± SEM; $P < 0.05$ or $p < 0.01$ was accepted as significant.

## RESULTS

**Effect of Anthocyanins on UVB-Mediated COX-2 Gene Expression and $PGE_2$ Production in HaCaT.** To investigate whether anthocyanins could exert an anti-inflammatory effect on UVB-damaged keratinocytes, inflammatory markers, specifically COX-2 involved in the UVB-induced inflammatory response, were determined after stimulation with UVB in HaCaT cells. UVB irradiation increased COX-2 expression at both the protein (**Figure 1A**) and the transcription activity levels (**Figure 1B**), which were inhibited by anthocyanins (**Figure 1A**,**C**). Moreover $PGE_2$, a product of COX-2, was increased by UVB irradiation and effectively diminished as the concentration of anthocyanins increased (**Figure 1D**).

**Effect of Anthocyanins on the UVB-Activated NF-$\kappa$B Pathway in HaCaT.** To determine if NF-$\kappa$B activated by UVB is inhibited by treatment with anthocyanins, NF-$\kappa$B translocation experiments and a luciferase reporter assay were performed. As shown in **Figure 2A**, UVB caused NF-$\kappa$B (p65) translocation from the cytosol to the nucleus. This effect was inhibited by pretreatment with anthocyanins in a concentration-dependent manner (**Figure 2A**). Phosphorylation of I$\kappa$B$\alpha$, an inhibitor of NF-$\kappa$B activity, by kinases results in the degradation of I$\kappa$B and subsequent release of NF-$\kappa$B, which translocates to the nucleus where it is active in regulation of gene transcription. Thus, it was determined if anthocyanins prevent phosphorylation of I$\kappa$B$\alpha$ by UVB. UVB irradiation induced I$\kappa$B$\alpha$ phosphorylation, which was dose dependently prevented by anthocyanins (**Figure 2A**). Moreover, UVB-increased NF-$\kappa$B-luciferase activity was approximately 4-fold higher as compared to untreated control (**Figure 2B**). However, when the mutant pcDNA/I$\kappa$B$\alpha$-SR was transfected, it did not change the luciferase activity induced by UVB (**Figure 2C**). In addition, anthocyanins efficiently inhibited NF-$\kappa$B-luciferase activity by UVB (**Figure 2D**). These results suggest that anthocyanins inhibit NF-$\kappa$B activation by UVB through prevention of I$\kappa$B$\alpha$ phosphorylation.

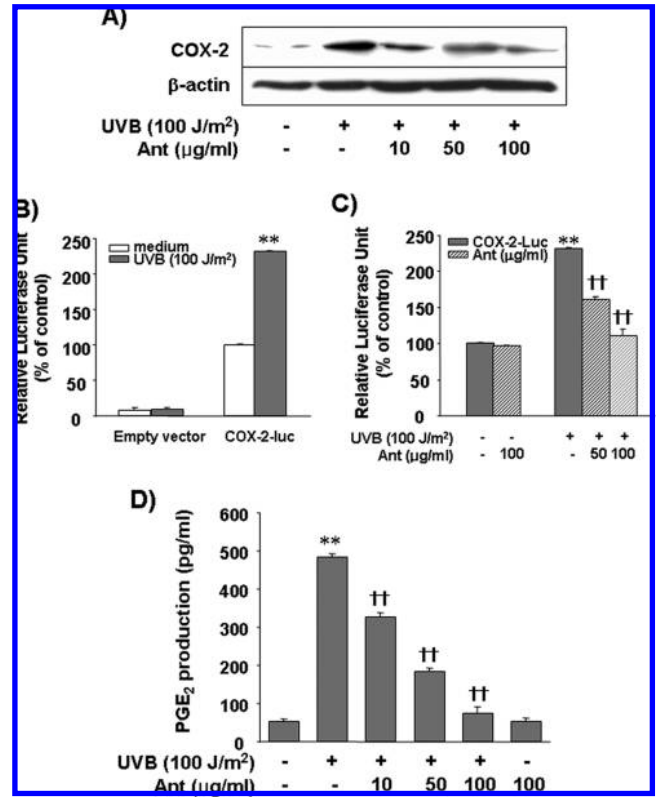


**Figure 1.** Effect of anthocyanins on UVB-mediated COX-2 gene expression and $PGE_2$ production in human keratinocyte cell line, HaCaT. (**A**) Cells were pretreated with anthocyanins (10, 50, and 100 $\mu$g/mL) for 24 h and then stimulated with UVB irradiation (100 J/m$^2$). Thereafter, media were replaced with fresh media containing anthocyanins, and cells were further incubated for 16 h. The COX-2 protein level was determined by Western blot analysis. The results were confirmed by three experiments ($n = 3$). (**B** and **C**) Cells were transiently cotransfected with empty vector or COX-2 promoter-luciferase + pRL-TK-luciferase. Transfected cells were pretreated with anthocyanins and stimulated with UVB irradiation as described in panel **A**. After further incubation with anthocyanins for 16 h, luciferase activities were determined from cell lysates and presented as percentage activation relative to untreated cells. The results were confirmed by three experiments ($n = 6$). (**D**) Cells were pretreated with anthocyanins and stimulated with UVB irradiation as described in panel **A**. After further incubation for 24 h, the supernatant was collected, and $PGE_2$ production was determined as described in the Materials and Methods. The results were confirmed by three experiments ($n = 6$). Significance as compared with the control, $^{**}p < 0.01$; significance as compared with UVB, $^{\dagger\dagger}p < 0.01$ (control level = 1).

**Differential Role of MAPK or PI3 Kinase/Akt in UVB-Induced COX-2 Expression and $PGE_2$ Production in HaCaT.** We investigated the signal transduction pathway that mediates increased COX-2 expression by UVB. Increased COX-2 protein levels after UVB irradiation were significantly diminished following addition of the specific ERK1/2 inhibitor, PD98059 (40 $\mu$mol/L), the P38 inhibitor, SB203580 (5 $\mu$mol/L), and the PI3 kinase inhibitor, LY294002 (5 $\mu$mol/L). In contrast, pretreatment with SP600125, a specific inhibitor of JNK, had no effect on UVB-induced COX-2 expression (**Figure 3A**). The production of $PGE_2$ by UVB was also inhibited by PD98059, SB203580, and LY294002, but not SP600125 (**Figure 3B**). This result indicates that the

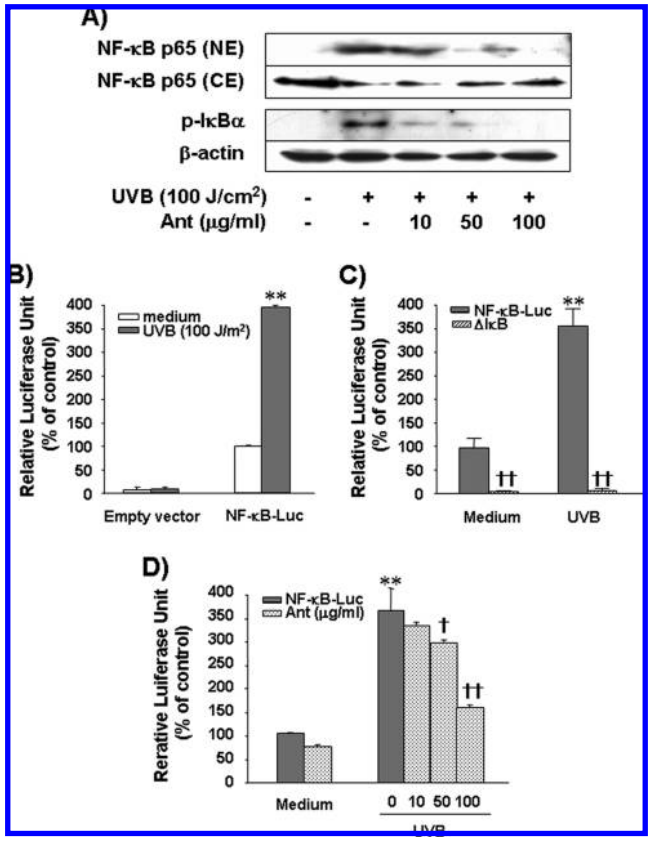


**Figure 2.** Inhibition of UVB-induced NF-$\kappa$B translocation into the nucleus, I$\kappa$B$\alpha$ phosphorylation, and NF-$\kappa$B-luciferase activity by anthocyanins in HaCaT. (**A**) Cells were pretreated with anthocyanins for 24 h and then stimulated with UVB irradiation. After 1 h of incubation with anthocyanins, nuclear and cytoplasmic fractions or total cell lysates were extracted, and the protein level was determined by Western blot analysis. Data were confirmed from two independent experiments ($n = 2$). (**B**−**D**) Cells were transfected with 1 $\mu$g of NF-$\kappa$B-luciferase, allowed to recover for 24 h, and then stimulated with UVB. After irradiation, cells were incubated in the presence or absence of anthocyanins. Cells were harvested 6 h after treatment, and luciferase activities were determined. The results were confirmed by three experiments ($n = 6$). Significance as compared with control, $^{**}P < 0.01$; significance as compared with UVB, $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$.

mechanism of COX-2 induction by UVB is differentially implicated in the MAPK pathway. Among them, ERK and p38, but not JNK, are involved in UVB-mediated induction of COX-2 gene expression and $PGE_2$ production.

**Effect of Anthocyanins on UVB-Induced Mitogenic Signaling in HaCaT.** UVB-induced phosphor-Akt was inhibited by anthocyanins in concentration-dependent manners. However, phosphor-MAPKs (ERK1/2, p38, and JNK) were not significantly influenced by anthocyanins (**Figure 4**). These results suggest that anthocyanins modulate UVB-induced COX-2 expression and $PGE_2$ production through regulation of the PI3 kinase/Akt signaling pathways activated by UVB irradiation. However, anthocyanins had little effect on the UVB-activated MAPK (ERK1/2, p38, or JNK) pathways.

**Effect of Anthocyanins on the Production of COX-2 and $PGE_2$ in UVB-Irradiated Mouse Skin.** To further confirm the effect of anthocyanins on COX-2 protein and $PGE_2$ production in vivo, we examined COX-2 protein levels and $PGE_2$ production in the skin of hairless mice irradiated with UVB (2.4 kJ/m$^2$). As expected, anthocyanins reduced COX-2 protein induction (**Figure 5A**) and $PGE_2$ production (**Figure 5B**) dose dependently in UVB-irradiated mouse skin.

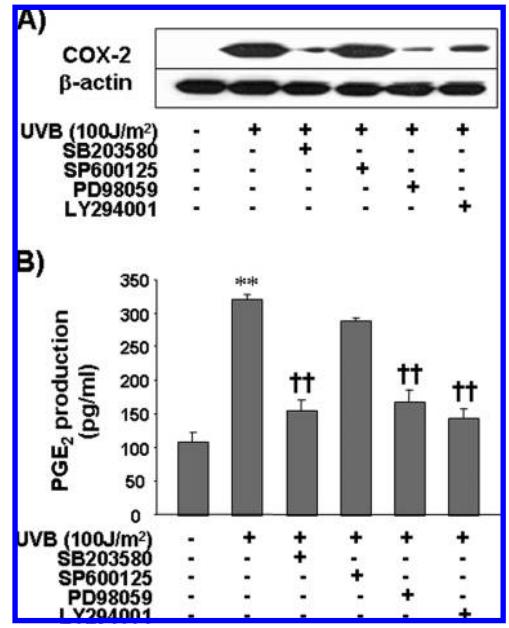


**Figure 3.** Signaling pathway involved in COX-2 expression and $PGE_2$ production by UVB in HaCaT. Cells were pretreated with PD98059 (an ERK1/2 inhibitor, 40 $\mu$mol/L), SB203580 (a p38 inhibitor, 5 $\mu$mol/L), SP600125 (a JNK inhibitor, 50 $\mu$mol/L), or LY294002 (a PI3 kinase inhibitor, 10 $\mu$mol/L) and then irradiated with UVB (100 J/m$^2$). The COX-2 protein level (**A**) or $PGE_2$ production (**B**) was detected 16 or 24 h after UVB irradiation, respectively. The results were confirmed by three experiments ($n = 3$ for **A**, and $n = 6$ for **B**, respectively). Significance as compared to the control, $^{**}p < 0.01$; significance as compared with UVB, $^{\dagger\dagger}p < 0.01$ (control level = 1).

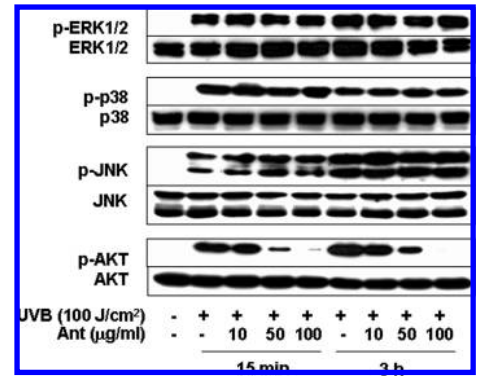


**Figure 4.** Effect of anthocyanins on UVB-mediated mitogenic signaling pathways (MAPKs and Akt) in HaCaT. To determine the effect of anthocyanins on the UVB-activated signaling pathway, cells were pretreated with anthocyanins at various concentrations (10, 50, or 100 $\mu$g/mL) for 24 h and then irradiated with UVB. At 15 min or 3 h of incubation with anthocyanins after UVB irradiation, phosphor-MAPKs and total-MAPK or phosphor-Akt and total-Akt protein levels were determined by Western blot analysis. Data were confirmed by three independent experiments ($n = 3$).

## DISCUSSION

The present study showed that novel anthocyanins from black soybean [*G. max* (L.) Merr] protected keratinocytes from UVB-mediated inflammation and abnormal survival signal, which was shown as inhibition of COX-2 expression and $PGE_2$ production and regulation of NF-$\kappa$B and PI3 kinase activation by UVB.

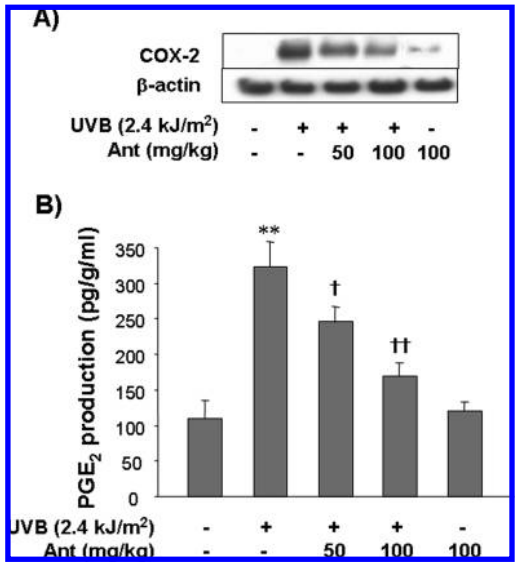


**Figure 5.** Effect of anthocyanins on COX-2 expression (**A**) and $PGE_2$ production (**B**) in UVB-irradiated hairless mouse skin. Fifteen hairless mice were divided into five groups of three animals each and exposed to UVB irradiation (2.4 kJ/m$^2$). Immediately after UVB irradiation, anthocyanins (50 or 100 mg/kg) were applied to mice. The dorsal skin was excised from each mouse, and Western blotting (**A**) and ELISA assay (**B**) were performed. The results were confirmed by three experiments ($n = 9$). Significance as compared with the control, $^{**}P < 0.01$; significance as compared with UVB irradiation, $^{\dagger\dagger}P < 0.01$.

The effect of anthocyanins on COX-2 expression and $PGE_2$ production was confirmed in hairless mice.

Recent studies have demonstrated that UVB-induced reactive oxygen species (ROS) cause inflammation and act as tumor promoters by a number of mechanisms including DNA damage, lipid peroxidation, and alteration of enzyme activity (*28*−*30*). Exposure of mammalian cells to UVB stimulates signal transduction pathways that typically culminate in activation of NF-κB, a redox-regulated transcription factor that is involved in the expression of a wide variety of proinflammatory genes including COX-2 (*31, 32*). All of the activation mechanisms that lead to IκB-α degradation and NF-κB translocation have been suggested to involve ROS (*33, 34*). NF-κB is upregulated by UVB in human skin in vivo (*35*) and HaCaT human keratinocytes in culture (*36, 37*). UVB-induced COX-2 expression is associated with erythema and infiltration of inflammatory cells such as mononuclear cells and neutrophils into the skin (*14, 38*). Moreover, it has been suggested that UVB irradiation-induced COX-2 gene expression and the resulting synthesis of PGs might contribute to UV radiation-induced skin carcinogenesis (*9*−*13*). Because of its specific upregulation in the epidermis during a chronic exposure to UVB radiation, COX-2 levels may actually be used as an early marker for potential skin tumorigenesis (*9, 11*). Therefore, COX-2 is a promising target for preventing photoinflammation and skin damage such as photoaging and tumor formation.

Exposure of cells to UV irradiation elicits a complex set of acute cellular responses known as "UV responses". These responses are mediated by a membrane-associated component of the Ras pathway and subsequent activation of the MAP kinases (*39*−*43*). Erks are involved in survival signaling in response to a variety of growth factors (*44, 45*), whereas activation of JNKs or p38 kinase is suggested to play decisive roles in the control of cell death (*45*). UV-generated ROS can also activate diverse downstream signaling molecules MAPK and NF-κB, as well as JNK and p38 MAPK. However, there are some differences between these reports and this study. In this study, although p38 and ERK1/2 of MAPK and PI3 kinase/Akt were involved in COX-2 and $PGE_2$ production by UVB, anthocyanins only regulated PI3 kinase/Akt pathway components activated by UVB irradiation. Whereas JNK was activated by UVB, JNK was not involved in UVB-mediated COX-2 and $PGE_2$ production. PI3 kinase/Akt pathway is also known as a mitogenic pathway. Actually, ionizing or UV radiation induced cellular survival signaling pathways protect cells from apoptosis; however, at the same time, it may induce development of cancer (*46*). Thus, it is important to maintain a balance between apoptosis and survival. As well as reduction of inflammatory COX-2 expression and $PGE_2$ production, anthocyanins from black soybean seed coats inhibited UVB-induced apoptotic cell death through regulation of caspase-3 activation and Bax/Bcl-2 ratio according to our unpublished data. Thus, in this respect, anthocyanins from black soybean seed coats may be useful to modulate UVB-induced harmful responses.

## ABBREVIATIONS USED

ANOVA, analysis of variance; COX-2, cylooxygenase-2; DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemoluminescence; ERK, extracellular regulated kinase; FBS, fetal bovine serum; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; TBS-T, Tris-buffered saline/Tween 20; UV, ultraviolet; PI3K, phosphatidylinositol 3-kinase; PG, prostaglandins.

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**Received for review April 30, 2008. Revised manuscript received July 7, 2008. Accepted August 13, 2008. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-311-E00392) and by ARPC (Agricultural R&D Promotion Center) (307003-03-1-HD140), Republic of Korea.**

JF801345C