Food Sci. Biotechnol. 22(2): 507-513 (2013)
DOI 10.1007/s10068-013-0108-7

RESEARCH ARTICLE

Cyanidin-3-*O*-(2''-xylosyl)-glucoside, an Anthocyanin from Siberian ginseng (*Acanthopanax senticosus*) Fruits, Inhibits UVB-induced COX-2 Expression and AP-1 Transactivation

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Received: 12 July 2012 / Revised: 17 October 2012 / Accepted: 17 October 2012 / Published Online: 30 April 2013

Abstract Cyanidin-3-*O*-(2''-xylosyl)-glucoside (C-3-*O*-(2''-xylosyl)-G) was analyzed as an active constituent from the fruit of Siberian ginseng (*Acanthopanax senticosus*) using a HPLC diode array detection-electrospray ionization/mass spectrometry analysis system and the effect of C-3-*O*-(2''-xylosyl)-G on UVB-induced inflammatory signaling in JB6 P+ cells was investigated. C-3-*O*-(2''-xylosyl)-G inhibited UVB-induced cyclooxygenase (COX)-2 expression and promoter binding activity in JB6 P+ cells and JB6 P+ cells stably transfected with the COX-2 luciferase reporter plasmid. It inhibited both the UVB-induced activator protein-1 (AP-1) and nuclear factor (NF)-κB transactivation in JB6 P+ cells stably transfected with the AP-1 and NF-κB luciferase reporter plasmids. Additionally, C-3-*O*-(2''-xylosyl)-G significantly suppressed UVB-induced upregulated phosphorylation of c-Jun N terminal kinase, MEK/extracellular signaling kinase, and mitogen activated protein kinase kinase (MAPKK) 3/6 in JB6 P+ cells. These results indicate that C-3-*O*-(2''-xylosyl)-G may be a promising chemopreventive material that acts by suppressing COX-2 expression and AP-1 and NF-κB transactivation and JNK, MAPKK3/6, and MEK/ERK1/2 phosphorylation.

Keywords: cyanidin-3-*O*-(2''-xylosyl)-glucoside, *Acanthopanax senticosus*, COX (cyclooxygenase)-2, activator protein-1 (AP-1), nuclear factor (NF)-κB

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Introduction

The bark and leaves of Siberian ginseng (*Eleutherococcus senticosus*, formerly *Acanthopanax senticosus*) have been used as a tonic and anti-stress drug (1). Multiple studies have shown that Siberian ginseng has biological functions including anti-oxidant and anti-inflammation properties as well as relieving anti-neuritic atrophy and synaptic loss effects (2-4). Cyanidin and its glycosides are mainly derived from fruits and red wines and are widely consumed by humans (5). Cyanidin-3-*O*-glucoside is a flavonoid and a member of the anthocyanin family, the largest group of pigments present in berries, cabbages, and wines. A previous study identified acylated anthocyanin, as cyaniding 3-[6-(*p*-coumaroyl)-2-(xylosyl)-glucoside]-5-glucoside and other anthocyanins from *Sambucus canadensis* (6). However, the chemopreventive effects and mechanisms of action of Cyanidin-3-*O*-(2''-xylosyl)-glucoside (C-3-*O*-(2''-xylosyl)-G) as non-acylated anthocyanin derived from Siberian ginseng fruit are unknown.

Cyclooxygenase (COX-2), a rate-limiting enzyme for the oxidative conversion of arachidonic acid into bioactive lipids including prostaglandins (PG), prostacyclins, and thromboxanes, has 2 main isoforms COX-1 and COX-2 (7,8). Chronic and acute UV irradiation induce COX-2

expression and its upregulation results in skin cancer (2,9,10). Treatment of mouse skin with a COX-2 inhibitor including non-steroidal anti-inflammatory drugs or celecoxib suppresses UV-induced skin cancer development by inhibiting COX-2/PGE2 expression (8,11). Additionally, a heterozygous *cox-2* null mouse study showed that COX-2 null mice do not form tumors (12). We also found that downregulation of UVB-induced COX-2 expression is a promising strategy to prevent skin carcinogenesis using natural phytochemicals (2,13).

Mitogen-activated protein kinases (MAPKs) play a central role in cell signaling, proliferation, and differentiation (9,14). The C-Jun N-terminal kinases 1/2 (JNK1/2), extracellular signal-regulated kinase 1/2 (ERK1/2), and p38 kinases are the 3 most studied subgroups of MAPKs. These 3 MAPKs are activated by MAPK kinases (MAPKKs) such as MKK4/7, MEK1/2, and MKK3/6 in response to UV exposure (9). Activated MAPKs increase transcriptional activity by activating transcription factors including activating protein-1 (AP-1) and nuclear factor-κB (NF-κB) (9). In previous study, we showed that UVB irradiation increases MAPKs including JNK1/2, ERK1/2, and p38 kinases in JB6 P+ cells (2). Other studies have shown that UV-induced AP-1 and NF-κB activity is involved in tumorigenesis (2,15). Furthermore, both AP-1 and NF-κB are closely related with COX-2 expression because COX-2 is a major AP-1 and NF-κB target gene (15).

This study has focused on identifying the effective chemo-preventive agents from the fruit of Siberian ginseng and investigating their biological effects. Here, it is reported that C-3-*O*-(2"-xyloxyl)-G, identified from the fruit of Siberian ginseng, inhibits UVB-induced COX-2 expression as well as COX-2, AP-1, and NF-κB transactivation and suppresses the UVB-induced phosphorylation of MKK4/JNK1/2, MEK1/2, and MKK3/6. These results indicate that C-3-*O*-(2"-xyloxyl)-G is a potent chemopreventive agent.

Materials and Methods

Materials Eagle's minimum essential medium (MEM), gentamicin, and L-glutamine were obtained from BD (Carlsbad, CA, USA). Fetal bovine serum (FBS) was purchased from Gemini Bio-Products (Calabasas, CA, USA). Antibodies to detect phosphorylated p38 (Tyr180/Tyr182), total p38, phosphorylated JNK (Thr183/Tyr185), total JNK, phosphorylated p90RSK (Thr359/Ser363), and total p90RSK were purchased from Cell Signaling Tchnology (Beverly, MA, USA). Antibodies against phosphorylated ERK1/2 (Thr202/Tyr204) and total ERK were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The active ERK protein was obtained from Upstate Biotechnology (Lake Placid, NY, USA). ATP and the chemiluminescence detection kit were purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA), and the protein assay kit was obtained from Bio-Rad Laboratories (Hercules, CA, USA). G418 and the luciferase assay substrate were purchased from Promega (Madison, WI, USA). Methanol, acetonitrile, formic acid, and water were purchased from Merck Chemical Co. (Darmstadt, Germany). Tetramethylsilane (TMS), CD_3OD, and DCl were obtained from Sigma-Aldrich (St. Louis, MO, USA). A 0.45-μm membrane filters were purchased from Waters Co. (Milford, MA, USA). All laboratory chemicals used in this study were of reagent grade.

Extraction and identification Fully matured Siberian ginseng (*Acanthopanax senticosus*) fruit was harvested in Samcheok, Korea. The air-dried whole Siberian ginseng (200 g) fruit was extracted with 1% HCl/20% CH_3OH (8 L) at 4°C for 48 h. The extracts were filtered through Advantec Toyo no. 2 filter paper and concentrated at 30°C *in vacuo*. The crude anthocyanin extracts was fractionated by semi-preparative HPLC with monitoring at 530 nm using a 250×9.4 mm i.d. Zorbax SB-C_{18} column (Agilent Technologies, Wilmington, DE, USA) at a column temperature of 30°C. Gradient elution was performed with solvent A, consisting of 5% aqueous formic acid, and solvent B, comprising 5% formic acid/acetonitrile, and delivered at a flow rate of 3 mL/min as follows: 0-10 min, 10-18% B; 10-18 min, 18-28% B; 18-19 min, 28-40% B; 19-21 min, 40% B; 21-23 min, 40-10% B; 23-25 min, 10% B. A sample volume of 2 mL was used for injection.

Instrumentation and conditions ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured on a Varian Unity Plus 500 NMR instrument in 0.5% DCl/CD_3OD containing TMS as the internal standard (Varian, Palo Alto, CA, USA). HPLC-electro spray ionization (ESI)-MS analysis of purified anthocyanin, was performed with a quaternary gradient pump, series 1200 from Agilent coupled to a Bruker Esquire LC-ESI-MS system (Bremen, Germany). The flow rate of the drying gas was 11.0 L/min, and nebulizer pressure was set to 413.7 kPa. The ESI-MS parameters were: capillary, 2,500 V; end plate, 3,000 V; capillary exit, +95 V; skim 1, +25 V; skim 2, +10 V; fragmentation amplitude, 0.8 V. HPLC separation was performed on a octadecyl saline (ODS) 120T 5 μm, 250×4.6 mm column (Tosoh, Tokyo, Japan). Gradient elution was carried out with the solvent system A (5% aqueous formic acid), and system B (5% formic acid/acetonitrile) at a flow rate of 0.7 mL/min. The gradient steps were set from 0-10 min, 10-18% B; 10-18 min, 18-28% B; 18-19 min, 28-40% B; 19-21 min, 40% B; 21-23 min, 40-10% B; 23-25 min, 10% B.

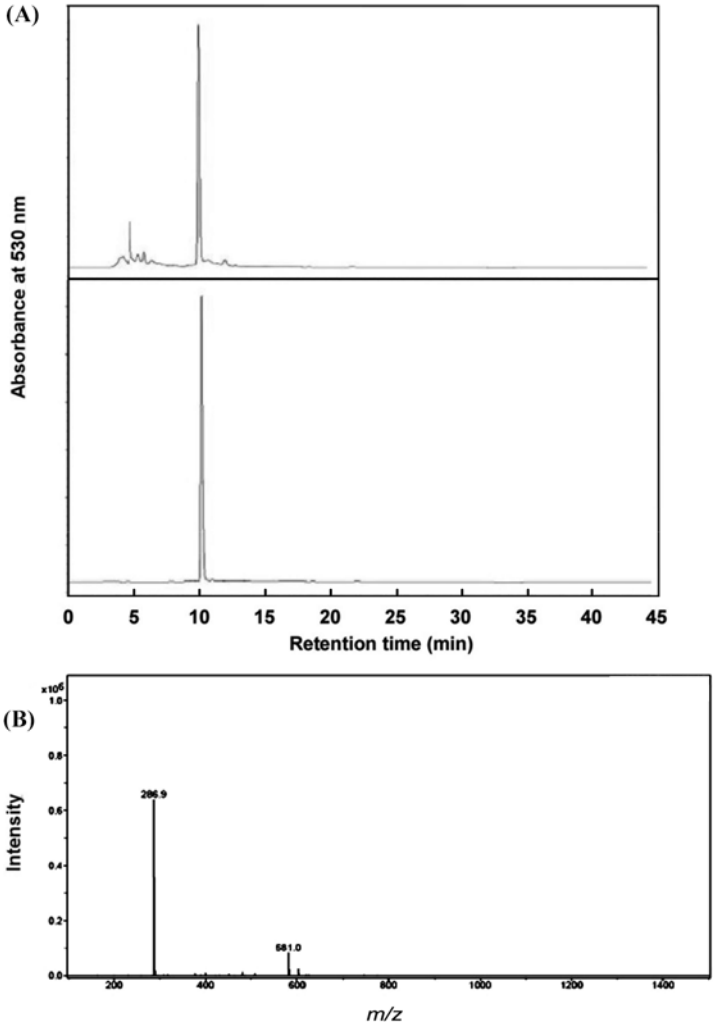

Fig. 1. HPLC (A) and LC-ESI-MS (B) chromatogram of the fruit extract and purified anthocyanin from Siberian ginseng fruit (A).

Cell culture The JB6 P+ mouse epidermal cell line was cultured in monolayers at 37°C in a 5% CO_2 incubator in 5% FBS-MEM, 2 mM L-glutamine, and 25 μg/mL gentamicin. The JB6 P+ mouse epidermal cell line was stably transfected with the AP-1 luciferase reporter plasmid and maintained in 5% FBS-MEM containing 200 μg/mL G418.

UVB irradiation A UVB irradiation system was used to stimulate cells in serum-free media. The spectral peak of the UVB source (Bio-Link Crosslinker, Vilber Lourmat, Torcy, France) was at 312 nm. The cells were exposed to UVB at 0.5 kJ/m^2 and then cultured for either 15 min or 6 h.

Luciferase assay for AP-1 and NF-κB transactivation Confluent monolayers of JB6 P+ cells stably transfected with the AP-1 and NF-κB luciferase plasmids were harvested, and 8×10^3 viable cells suspended in 10 mL 5% FBS/MEM were added to each well of a 96-well plate. The plates were incubated at 37°C in a 5% CO_2 incubator. When the cells reached 80-90% confluence, they were starved by culturing in 0.1% FBS-MEM for another 24 h. The cells were treated with C-3-*O*-(2"-xyloxyl)-G (10 and 20 μg/mL) 1 h before exposure to UVB (0.05 J/cm^2) and then were incubated for 6 h. The cells were disrupted with 100 μL lysis buffer [0.1 M potassium phosphate buffer (pH 7.8), 1% Triton X-100, 1 mM dithiothreitol, and 2 mM EDTA] and luciferase activity was measured using a luminometer (Luminoskan Ascent; Thermo Electron, Helsinki, Finland).

Western blot assays Cells (1.5×10^6) were cultured in a 10-cm dish for 48 h, and then starved in 0.1% FBS-MEM for 24 h to eliminate FBS activation of MAPKs. Then the cells were treated with C-3-*O*-(2"-xyloxyl)-G (10 and 20 μg/mL) for 1 h and irradiated with UVB (0.05 J/cm^2). Protein concentration was determined using a Dye-binding Protein assay kit (Bio-Rad Laboratories), as described in the manufacturer's manual. Lysate protein (40 μg) was subjected to 10% SDS-PAGE and transferred to a poly-

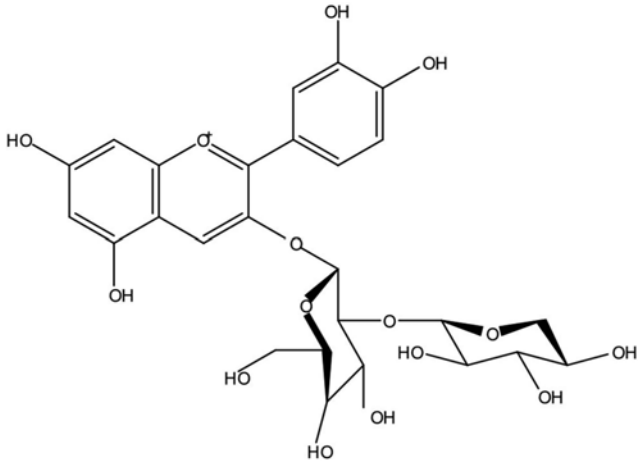

Fig. 2. Structure of cyanidin-3-*O*-(2''-xylosyl)-glucoside.

vinylidene difluoride membrane (Amersham Pharmacia Biotech). After transfer, the membrane was incubated with the specific primary antibody at 4°C overnight. Protein bands were visualized by a Chemiluminescence detection kit (Amersham Pharmacia Biotech) after hybridization with a horseradish peroxidase-conjugated secondary antibody. The relative amounts of proteins associated with specific antibodies were quantified using Scion Image (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis Data are expressed as means± standard error (SE), and significant differences were determined using a one-way analysis of variance (ANOVA). A p-value<0.05 was considered significance.

Results and Discussion

Isolation and identification of purified anthocyanin from Siberian ginseng Chemoprevention, the use of drugs or natural substances to retard or reverse carcinogenesis, is a promising strategy for reducing the risk of cancer development (16,17). In our previous studies, we have shown that natural phytochemicals act as chemopreventive agents via the suppression of oncogenic signaling pathways (2,18). Among natural chemopreventive candidates, the bark and leaves of Siberian ginseng have antioxidant, anti-inflammation, and anti-cancer effects (18-20). However, the major components of the fruit of Siberian ginseng and their chemopreventive effects remain unclear. To identify the active compounds from the fruit of Siberian ginseng, the fruit extract was isolated using semi-preparative HPLC techniques with an RP-18 column. Purified anthocyanin was identified using HPLC-diode array detection-ESI/MS analysis based on the profiles obtained from the ^{1}H and ^{13}C NMR spectroscopic data. Structural confirmation of purified anthocyanin was obtained from heteronuclear correlation experiments (HMQC, HMBC), and cyanidin was corroborated as the anthocyanin moiety in the structure. The cross signal from the H-1'' of the glucose δ=5.43 to C-3 δ=145.5 ppm clearly elucidated the proposed glycosidation to the cyanidin backbone. The disaccharide linkage glucose (2→1) xylose was confirmed by the correlation peak H-1''' of the xylose at δ=4.72 ppm to glucose carbon C-2'' at δ=81.39 ppm, and also with a significant glycosidation shift downfield. The ^{13}C NMR data for the disaccharide moieties of purified anthocyanin were almost identical (Table 1), confirming the 3-(2''-xylosyl) glucoside structure. Typical coupling constants elucidated the glycosidic linkages: J=7.5 Hz for the anomer H-1'' at δ=5.43 ppm determined the β-glucosidic linkage to the aglycone moiety, and a similar value of J=7.5 Hz observed for the anomer H-1''', β-linked xylopyranoside unit. The positive ESI-MS molecular ions (m/z) were 581 $[M+H]^+$, and 287 $[M\text{-glucose-xylose}]^+$ for the cyanidin aglycone. The neutral loss m/z=294 confirmed the cleavage of the disaccharide unit 3-(2''-xylosyl) glucoside as established by previous literature (21). Therefore, the purified anthocyanin of Siberian ginseng was identified cyanidin-3-*O*-(2''-xylosyl)-G. A large number of experimental studies have identified more than 16 terpenoid saponins in Siberian ginseng fruit and have demonstrated its biological effects (22,23). However, no reports have identified C-3-*O*-(2''-xylosyl)-G as a major constituent in the fruit of Siberian ginseng.

C-3-*O*-(2''-xyloxyl)-G suppresses UVB-induced COX-2 expression and promoter binding activity Many studies have suggested that COX-2 is involved in tumorigenesis (2,8,11,12). A genetically modified *cox-2* null mice model showed that COX-2 plays a critical role in tumor promotion (12). In this study, we adopted the JB6 P+ and UVB model

Table 1. ^{1}H- and ^{13}C-NMR spectral data of the purified anthocyanin in Siberian ginseng fruit

	Purified anthocyanin	
	^{13}C-NMR	^{1}H-NMR δ (ppm) *J* (Hz)
Carbon		
2	164.41	
3	145.51	
4	133.54	8.96 s
5	159.26	
6	103.27	6.67 d 2
7	170.37	
8	97.68	6.92 d 1.5
9	157.61	
10	113.30	
1'	121.40	
2'	118.66	8.05 d 2.5
3'	147.53	
4'	155.89	
5'	117.50	7.03 d 9
6'	128.81	8.30 dd 2, 9
3-*O*-β-D-Glucoside		
1"	102.17	5.43 d 7.5
2"	81.39	3.98 dd 8, 9
3"	77.30	3.79 t, 9
4"	71.09	3.48 dd 9.5, 9.5
5"	77.92	3.61 ddd 6.5, 6, 2
6"	62.33	3.98 dd 2, 12 3.77 dd 6, 12
2"-*O*-Xyloside		
1	106.30	4.72 d 7.5
2	75.20	3.14 dd 8, 9
3	78.80	3.28 m
4	70.14	3.35 ddd 9, 10.5, 5.5
5	67.26	3.63 dd 5.5, 11.5 3.03 dd 10.5, 11.5

because this system is optimal for the study of chemopreventive effects of phytochemicals (2,13,24). We first investigated the inhibitory effect of C-3-*O*-(2"-xyloxyl)-G on UVB-induced COX-2 upregulation in JB6 P+ cells. As shown in Fig. 3A, UVB-induced COX-2 expression was inhibited by C-3-*O*-(2"-xyloxyl)-G. UVB-induced COX-2 promoter activity was determined to confirm the regulatory mechanism of C-3-*O*-(2"-xyloxyl)-G on UVB-induced COX-2 expression. C-3-*O*-(2"-xyloxyl)-G significantly inhibited UVB-induced COX-2 promoter activity in stably transfected *cox-2* promoter luciferase cells (Fig. 3B), indicating that COX-2 expression was regulated by C-3-*O*-(2"-xyloxyl)-G by mediating transcriptional regulation of the *cox-2* gene.

C-3-*O*-(2"-xyloxyl)-G reduces UVB-induced transactivation of AP-1 and NF-κB AP-1 and NF-κB are transcription factors implicated in cell proliferation,

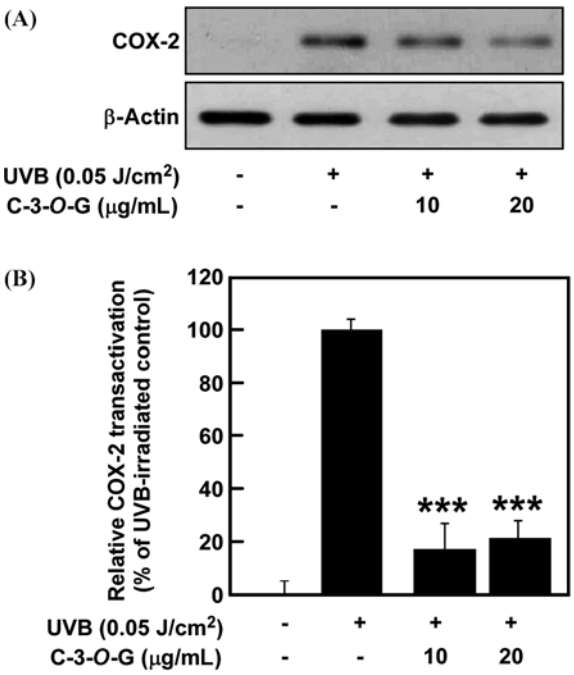

Fig. 3. Effects of C-3-*O*-(2"-xyloxyl)-G on UVB-induced COX-2 expression (A) and promoter activity (B) in JB6 P+ cells. A, data are representative of 3 independent experiments that produced similar results. B, COX-2 activity is expressed relative to that of control cells not exposed to UVB radiation. Data are mean±SD of COX-2 luciferase activity from 3 independent experiments; ***Indicate a significant difference at $p<0.001$ between groups treated with UVB and C-3-*O*-(2"-xyloxyl)-G and the group treated with UVB alone.

inflammation, transformation, and carcinogenesis (25,26). Suppression of AP-1 and NF-κB activation by a pharmaceutical inhibitor reduces carcinogen-induced neoplastic transformation (27). Furthermore, AP-1 and NF-κB activity is involved in COX-2 expression in MCF-7 breast cancer cells and the rat brain (28,29). These observations show that COX-2 expression is regulated by the AP-1 and NF-κB transcription factors. In our previous study, we demonstrated UVB-irradiation induced AP-1 and NF-κB transactivity and COX-2 expression in JB6 P+ cells (2). To determine the mechanism of transcriptional regulation of COX-2 by C-3-*O*-(2"-xyloxyl)-G, we evaluated the inhibitory activities of C-3-*O*-(2"-xyloxyl)-G on the UVB-induced transactivation of AP-1 and NF-κB luciferase activity, which are major transcriptional factors involved in COX-2 expression (25). The results showed that C-3-*O*-(2"-xyloxyl)-G significantly inhibited UVB-induced AP-1 and NF-κB transactivation in stably transfected AP-1 and NF-κB luciferase reporter JB6 P+ cells (Fig. 4A, 4B). A previous study also showed that either anthocyanin, or cyaniding-3-glucoside, strongly suppressed tumor growth *in vivo* via the suppression of UVB and TPA-induced AP-1 and transactivation (24). This result indicated that C-3-*O*-(2"-xyloxyl)-G could be effective chemopreventive agent

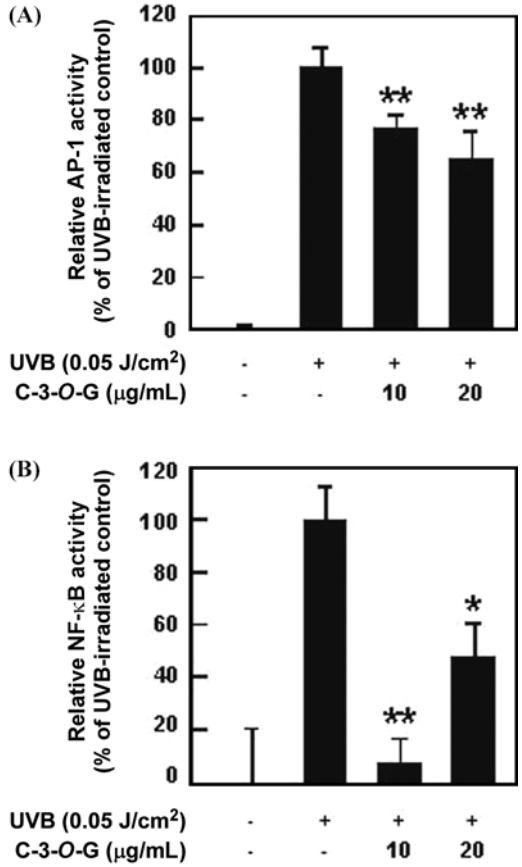

Fig. 4. Effects of C-3-*O*-(2''-xyloxyl)-G on UVB-induced AP-1 (A) and NF-κB (B) transactivation in JB6 P+ cells. Data are represented as the mean±SD of AP-1 and NF-κB luciferase activity calculated from 3 independent experiments; Significant differences at $*p<0.05$ and $**p<0.01$, respectively, between groups treated with UVB and C-3-*O*-(2''-xyloxyl)-G and the group treated with UVB alone

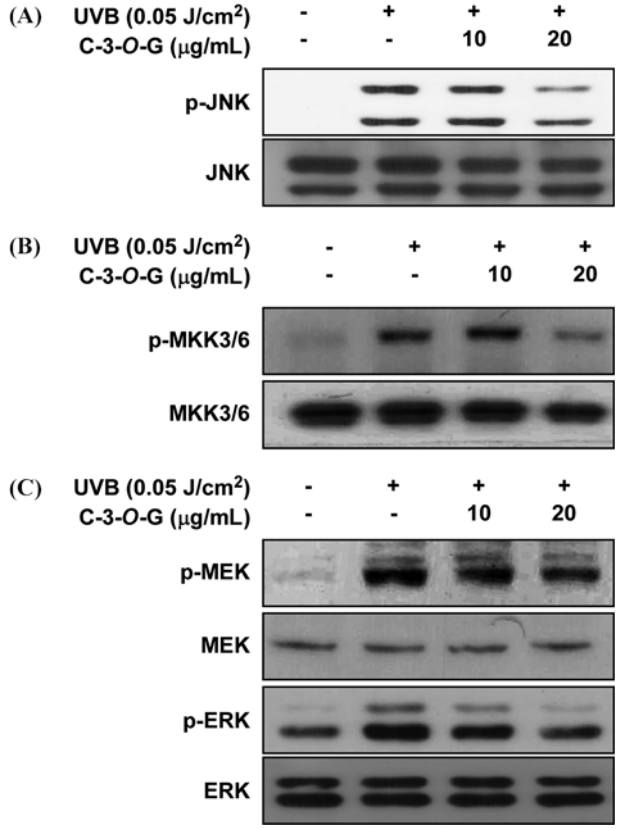

Fig. 5. Effects of C-3-*O*-(2''-xyloxyl)-G on UVB-induced MAPKs signaling in JB6 P+ cells. C-3-*O*-(2''-xyloxyl)-G inhibited UVB-induced phosphorylation of JNK1/2 (A), MKK3/6 (B), and MEK and ERK1/2 (C) in JB6 P+ cells. Data are representative of 3 independent experiments that produced similar results.

because it significantly suppressed UVB-induced AP-1 and NF-κB transactivation.

C-3-*O*-(2''-xyloxyl)-G inhibits UVB-induced phosphorylation of MEK/ERK, MKK4/JNK1/2, and MKK3/6

Many studies have shown that MAPKs regulate COX-2 expression through AP-1 and NF-κB transcriptional activity (9). Activated ERK signaling subsequently activates AP-1 activity by regulating the stability, of c-Fos, a subunit of AP-1 (30). JNK is a well known mediator of AP-1 and NF-κB activity. Therefore, the inhibition of MAPKs is an effective strategy to suppress COX-2 expression by the regulation of AP-1 and NF-κB activity. Thus, we evaluated the inhibitory effect of C-3-*O*-(2''-xyloxyl)-G on UVB-induced MAPKKs/MAPKs including MEK/ERK, MKK4/JNK, and MKK3/6 phosphorylation. Our Western blot assay results clearly showed that C-3-*O*-(2''-xyloxyl)-G significantly reduced UVB-induced MEK/ERK, MKK4/JNK, and MKK3/6 phosphorylation in JB6 P+ cells (Fig. 5). Further studies including a pull-down assay and an animal study are required to identify the direct target and molecular mechanism of C-3-*O*-(2''-xyloxyl)-G.

In conclusion, we have shown that C-3-*O*-(2''-xyloxyl)-G isolated from Siberian ginseng significantly inhibits UVB-induced JNK, MKK3/6, and MEK/ERK1/2 phosphorylation and AP-1 and NF-κB transactivation and subsequently COX-2 promoter binding activity and expression. We suggest that this C-3-*O*-(2''-xyloxyl)-G may be useful as a novel chemopreventive agent.

Acknowledgments This work was supported by a National Research Foundation of Korea (NRF) grant, funded by the Korean government (MEST; No. 2010-0029233) by Interrelated Development Program (R-0000451) of Inter-Economic Regions, Ministry for Knowledge Economy, Republic of Korea, and World Class University Program (Grant R31-2008-00-1-56-0).

References

1. Deyama T, Nishibe S, Nakazawa Y. Constituents and pharmacological effects of Eucommia and Siberian ginseng. Acta Pharmacol. Sin. 22: 1057-1070 (2001)
2. Jung SK, Lee KW, Byun S, Kang NJ, Lim SH, Heo YS, Bode AM, Bowden GT, Lee HJ, Dong Z. Myricetin suppresses UVB-induced skin cancer by targeting Fyn. Cancer Res. 68: 6021-6029 (2008)
3. Tohda C, Ichimura M, Bai Y, Tanaka K, Zhu S, Komatsu K. Inhibitory effects of *Eleutherococcus senticosus* extracts on amyloid β(25-35)-induced neuritic atrophy and synaptic loss. J. Pharm. Sci. 107: 329-339 (2008)
4. Yu CY, Kim SH, Lim JD, Kim MJ, Chung IM. Intraspecific relationship analysis by DNA markers and *in vitro* cytotoxic and antioxidant activity in *Eleutherococcus senticosus*. Toxicol. In Vitro 17: 229-236 (2003)
5. Galvano F, La Fauci L, Lazzarino G, Fogliano V, Ritieni A, Ciappellano S, Battistini NC, Tavazzi B, Galvano G. Cyanidins: Metabolism and biological properties. J. Nutr. Biochem. 15: 2-11 (2004)
6. Johansen OP, Andersen OM, Nerdal W, Aksnes DW. Cyanidin 3-[6-(*p*-coumaroyl)-2-(xylosyl)-glucoside]-5-glucoside and other anthocyanins from fruits of *Sambucus canadensis*. Phytochemistry 30: 4137-4141 (1991)
7. Fecker LF, Stockfleth E, Nindl I, Ulrich C, Forschner T, Eberle J. The role of apoptosis in therapy and prophylaxis of epithelial tumours by nonsteroidal anti-inflammatory drugs (NSAIDs). Brit. J. Dermatol. 156: 25-33 (2007)
8. Rao CV, Reddy BS. NSAIDs and chemoprevention. Curr. Cancer Drug Targets 4: 29-42 (2004)
9. Bode AM, Dong Z. Mitogen-activated protein kinase activation in UV-induced signal transduction. Sci. STKE 2003: RE2 (2003)
10. Rodust PM, Stockfleth E, Ulrich C, Leverkus M, Eberle J. UV-induced squamous cell carcinoma-A role for antiapoptotic signalling pathways. Brit. J. Dermatol. 161: 107-115 (2009)
11. Grau de Castro JJ. COX-2 inhibitors in cancer prevention. Rev. Clin. Esp. 205: 446-456 (2005)
12. Fischer SM, Pavone A, Mikulec C, Langenbach R, Rundhaug JE. Cyclooxygenase-2 expression is critical for chronic UV-induced murine skin carcinogenesis. Mol. Carcinogen. 46: 363-371 (2007)
13. Lee KM, Lee KW, Byun S, Jung SK, Seo SK, Heo YS, Bode AM, Lee HJ, Dong Z. 5-Deoxykaempferol plays a potential therapeutic role by targeting multiple signaling pathways in skin cancer. Cancer Prev. Res. 3: 454-465 (2010)
14. Bowden GT. Prevention of non-melanoma skin cancer by targeting ultraviolet-B-light signalling. Nat. Rev. 4: 23-35 (2004)
15. Lu H, Li J, Zhang D, Stoner GD, Huang C. Molecular mechanisms involved in chemoprevention of black raspberry extracts: From transcription factors to their target genes. Nutr. Cancer 54: 69-78 (2006)
16. Bode AM, Dong Z. Cancer prevention research-Then and now. Nat. Rev. 9: 508-516 (2009)
17. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat. Rev. 3: 768-780 (2003)
18. Wang X, Hai CX, Liang X, Yu SX, Zhang W, Li YL. The protective effects of *Acanthopanax senticosus* Harms aqueous extracts against oxidative stress: Role of Nrf2 and antioxidant enzymes. J. Ethnopharmacol. 127: 424-432 (2010)
19. Hibasami H, Fujikawa T, Takeda H, Nishibe S, Satoh T, Fujisawa T, Nakashima K. Induction of apoptosis by *Acanthopanax senticosus* HARMS and its component, sesamin in human stomach cancer KATO III cells. Oncol. Rep. 7: 1213-1216 (2000)
20. Kang OH, Choi YA, Park HJ, Kang CS, Song BS, Choi SC, Nah YH, Yun KJ, Cai XF, Kim YH, Bae KH, Lee YM. Inhibition of trypsin-induced mast cell activation by acanthoic acid. J. Ethnopharmacol. 105: 326-331 (2006)
21. Nayak CA, Srinivas P, Rastogi NK. Characterisation of anthocyanins from *Garcinia indica* Choisy. Food Chem. 118: 719-724 (2010)
22. Jin JL, Lee S, Lee YY, Kim JM, Heo JE, Yun-Choi HS. Platelet anti-aggregating triterpenoids from the leaves of *Acanthopanax senticosus* and the fruits of *A. sessiliflorus*. Planta Med. 70: 564-566 (2004)
23. Li F, Li W, Fu H, Zhang Q, Koike K. Pancreatic lipase-inhibiting triterpenoid saponins from fruits of *Acanthopanax senticosus.* Chem. Pharm. Bull. 55: 1087-1089 (2007)
24. Kang NJ, Lee KW, Rogozin EA, Cho YY, Heo YS, Bode AM, Lee HJ, Dong Z. Equol, a metabolite of the soybean isoflavone daidzein, inhibits neoplastic cell transformation by targeting the MEK/ERK/p90RSK/activator protein-1 pathway. J. Biol. Chem. 282: 32856-32866 (2007)
25. Ding M, Feng R, Wang SY, Bowman L, Lu Y, Qian Y, Castranova V, Jiang BH, Shi X. Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. J. Biol. Chem. 281: 17359-17368 (2006)
26. Eferl R, Wagner EF. AP-1: A double-edged sword in tumorigenesis. Nat. Rev. 3: 859-868 (2003)
27. Li JJ, Westergaard C, Ghosh P, Colburn NH. Inhibitors of both nuclear factor-κB and activator protein-1 activation block the neoplastic transformation response. Cancer Res. 57: 3569-3576 (1997)
28. Degner SC, Kemp MQ, Bowden GT, Romagnolo DF. Conjugated linoleic acid attenuates cyclooxygenase-2 transcriptional activity via an anti-AP-1 mechanism in MCF-7 breast cancer cells. J. Nutr. 136: 421-427 (2006)
29. Rao JS, Bazinet RP, Rapoport SI, Lee HJ. Chronic treatment of rats with sodium valproate downregulates frontal cortex NF-κB DNA binding activity and COX-2 mRNA. Bipolar Disord. 9: 513-520 (2007)
30. Okazaki K, Sagata N. The Mos/MAP kinase pathway stabilizes c-Fos by phosphorylation and augments its transforming activity in NIH 3T3 cells. EMBO J. 14: 5048-5059 (1995)