Ginsenoside F1 Protects Human HaCaT Keratinocytes from Ultraviolet-B-Induced Apoptosis by Maintaining Constant Levels of Bcl-2

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Ginsenosides, the major active ingredients of ginseng, show a variety of biomedical efficacies such as antiaging and antioxidation. Here, we investigate the protective activity of the ginsenoside F1, an enzymatically modified derivative of ginsenoside Rg1, against ultraviolet-B-induced damage in human HaCaT keratinocytes. Ginsenoside F1 significantly reduced ultraviolet-B-induced cell death and protected HaCaT cells from apoptosis caused by ultraviolet B irradiation. Furthermore, ginsenoside F1 prevented ultraviolet-B-induced cleavage of poly(ADP-ribose) polymerase in HaCaT cells. In search of the molecular mechanism responsible for the antiapoptotic effect of ginsenoside F1, we find that protection from ultraviolet-B-induced apoptosis is tightly correlated with ginsenoside-F1-mediated inhibition of ultraviolet-B-induced downregulation of Bcl-2 and Brn-3a expression. ***Key words: apoptosis/Bcl-2/ginsenoside F1/HaCaT/ultraviolet B. J Invest Dermatol 121:607–613, 2003***

Ultraviolet radiation (UV), in particular ultraviolet B (UVB) with a wavelength range between 290 and 320 nm, is one of the most important environmental factors because of its hazardous health effects, which include generation of skin cancer (De Gruijl *et al*, 1993), suppression of the immune system (Beissert and Schwarz, 1999), and premature skin aging (Fisher *et al*, 1997). One of the hallmark events of UV-exposed skin is the formation of apoptotic keratinocytes, which die as sunburn cells within the epidermis. Biochemical analyses reveal that UVB-irradiated keratinocytes show DNA fragmentation accompanied by sequential caspase activation (Takahashi *et al*, 1997).

The Bcl-2 protein is constitutively expressed in the human epidermis and regulation of its expression level may have an important role in the generation of sunburn cells (Hockenbery *et al*, 1991; Rodriguez-Villanueva *et al*, 1995; Sermadiras *et al*, 1997). Bcl-2 is an antiapoptotic protein that blocks apoptosis probably by inhibiting the channel-forming activity of proapoptotic family members like Bax (Adams and Cory, 1998). UVB irradiation has been known to decrease Bcl-2 expression without alteration of Bax expression in human keratinocytes (Gillardon *et al*, 1994). Furthermore, Bcl-2-transfected HaCaT cells or Bcl-2-overexpressing transgenic mice were shown to be resistant to UVB-induced apoptosis (Haake and Polakowka, 1995; Takahashi *et al*, 2001). Although these findings suggest that Bcl-2 is a key regulator in the apoptotic pathway of UVB-irradiated skin, little is known about the mechanisms governing the downregulation of Bcl-2 expression caused by UVB irradiation.

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Abbreviation: PARP, poly(ADP-ribose) polymerase.

Ginsenosides represent the major active ingredients of ginseng, which show a variety of biomedical efficacies, such as immune modulation, antiaging, anti-inflammation, and antioxidation in the central nervous, cardiovascular, endocrine, and immune systems (Wang *et al*, 2000; Yu and Li, 2000; Chen *et al*, 2001). More than 20 ginsenosides have been isolated and identified to date (Gillis, 1997). Ginsenoside Rg1 prevents rat cortical neurons from apoptosis (Li *et al*, 1997), whereas ginsenoside Rh2 inhibits growth in various cancer cells (Ota *et al*, 1987). The action mechanism and potential role of ginsenosides in keratinocytes, however, are poorly understood.

In this study we have investigated the role of a ginsenoside in UVB-induced apoptosis of keratinocytes. For the study, the ginsenoside F1 (20-O-β-D-glucopyranosyl-20 (S)-protopanaxatriol), an enzymatically modified molecule of ginsenoside Rg1, is used (**Fig 1**). We have shown that UVB irradiation downregulates Bcl-2 expression via a downregulation of Brn-3a transcription factor in HaCaT cells, and ginsenoside F1 protects these cells against UVB-induced apoptosis by maintaining constant levels of Brn-3a and the corresponding inhibition of Bcl-2 downregulation.

MATERIALS AND METHODS

Ginsenoside F1 preparation Ten grams of total ginseng extracts (red ginseng, white ginseng, tiny ginseng roots, and ginseng leaves) were dissolved in 2 liters of citrate buffer (pH 4.0) and incubated with 10 g of naringinase (Sigma St. Louis, MO) or 10 g of pectinase (Novozyme Krogshoejvej, Bagsvaerd) for 48 h at 38°C. When the enzymatic hydrolysis reaction was complete, the reaction mixture was extracted with 2 liters of ethyl acetate. After evaporation *in vacuo*, 2.8 g of the residue was obtained. To obtain pure ginsenoside F1, the resulting products were separated through silica gel column chromatography and eluted with chloroform:methanol (9:1). Further elution with chloroform:methanol (6:1) afforded the pure ginsenoside F1 (0.28 g). The identification was done by FAB-MS, 1H-NMR, and 13C-NMR spectra and the purity was

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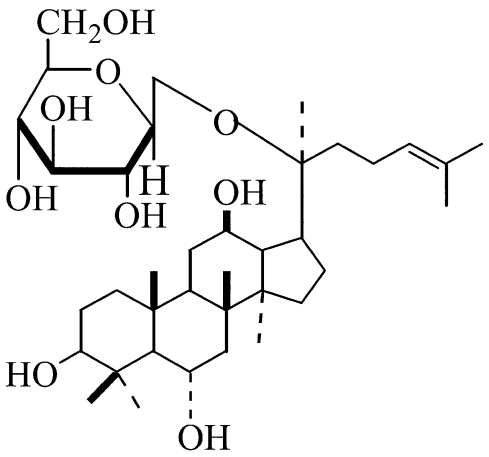


Figure 1. *Structure of ginsenoside F1.*

assessed by high performance liquid chromatography and determined to be about 96%. Ginsenoside F1 was dissolved in 100% ethanol to make a stock solution. Ethanol concentration was kept below 0.001% in all the cell cultures and did not exert any detectable change in cell growth or apoptosis.

Cell cuture and ginsenoside F1 treatment Human HaCaT keratinocytes were obtained from Dr Fusenig of the German Cancer Research Center (DKFZ) and the cells ($1.5–2 \times 10^5$ cells per well) placed in six-well plates were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum for 24 h. After serum starvation for 24 h, medium was replaced by serum-free medium with various concentrations of ginsenoside F1, and cells were incubated for 24 h prior to irradiation with UVB.

UVB irradiation Ginsenoside-F1-treated or untreated HaCaT cells were rinsed twice with PBS and exposed to various doses of UVB (0–120 mJ per cm^2), the intensity of which was monitored with an IL-1700 radiometer. A UVB radiation source was provided by a bank of Sankyo Denki G15T8E, a fluorescent bulb emitting 270–320 nm wavelength with a peak at 313 nm. Further, a Kodacel cellulose film (Kodacel TA401/407) was used to completely eliminate UVC radiation. After UVB exposure, cells were replenished with serum-free medium including ginsenoside F1 and followed up to 24 h.

Cell viability assay To determine cell viability, Trypan blue dye exclusion and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assays were performed at 24 h after treatment with different doses of ginsenoside F1 (1, 5, 10, 50 μm) in HaCaT cells as described by Fumelli *et al* (2000). The results of MTT assays were expressed as optical density units (OD) using an ELISA reader (Thermo Max, Molecular Devices, Sunnyvale, CA) at 540 nm. At 24 h after UVB irradiation (0–120 mJ per cm^2), the relative number of living cells was detected by MTT assay. The ratio of viable cells was expressed as a percentage of the OD value obtained without UVB treatment.

Analysis of DNA fragmentation At 24 h after UVB irradiation, ginsenoside-F1-treated and untreated HaCaT cells were analyzed by TUNEL staining and DNA fragmentation assay. TUNEL staining was performed with the TUNEL Apoptosis Detection Kit (Upstate, New York), as recommended by the manufacturer. The biotin-labeled nuclei cells were detected by fluorescein isothiocyanate (FITC) avidin. Nuclei were counterstained using propidium iodide and cells were analyzed under a fluorescence microscope. For analysis of DNA fragmentation, DNA was isolated from the cells as described by Baba *et al* (1998). Twenty microgram of DNA was subjected to 2% agarose gel electrophoresis.

RT-PCR mRNA was extracted from cultures using Oligotex Direct mRNA kits (Qiagen, Hilden, Germany), following the manufacturer's instructions. One microgram of mRNA was used as template for quantitative RT-PCR. cDNA synthesis and PCR amplification were performed using the Platinum Quantitative RT-PCR ThermoScript One-Step System (Invitrogen Grand Island, NY), according to the instructions of the manufacturer.

For specific human Bcl-2 or Bax amplification, the oligonucleotides 5′-TACGATAACCGGGAGATAGTGA-3′ (sense, bases 56–77 of human Bcl-2 cDNA) and 5′-CAGGTGCCGGTTCAGGTACT-3′ (antisense, bases 566–586 of human Bcl-2 cDNA) or the oligonucleotides 5′-CAAGAAGC TGAGCGAGTGTCT-3′ (sense, bases 220–240 of human Bax cDNA) and 5′-GGTTCTGATCAGTTCCGGCAC-3′ (antisense, bases 437–457 of human Bax cDNA) were used. For specific human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA amplification, the oligo-

Table I. Effect of ginsenoside F1 on the viability and cytotoxicity of HaCaT cells[a]

Ginsenoside F1	Trypan blue, % ± SD[b]	MTT, OD ± SD[b]
Control	92.73 ± 6.30	1.229 ± 0.03
1 μm	90.07 ± 2.89	1.204 ± 0.10
5 μm	91.14 ± 2.92	1.252 ± 0.09
10 μm	89.08 ± 3.00	1.210 ± 0.05
50 μm	56.45 ± 4.56	0.704 ± 0.10

[a]HaCaT cells were treated with the indicated concentration of ginsenoside F1 for 24 h.
[b]Data are mean ± SD of triplicate determinations.

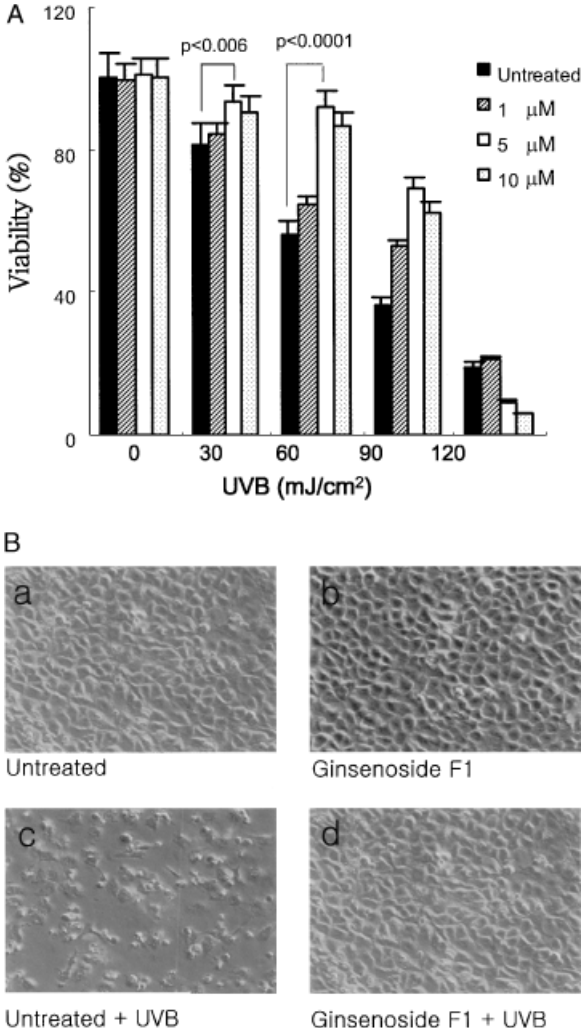


Figure 2. Ginsenoside F1 prevents UVB-induced cell death. (*A*) HaCaT cells were treated for 24 h with different doses of ginsenoside F1 (0, 1, 5, 10 μm) and were then exposed to UVB radiation with increasing intensities, as indicated. At 24 h after UVB irradiation, viability was assessed by MTT assay. Results are expressed as the mean ± SD of six different experiments. Student's *t* test was used for comparison of the means. (*B*) HaCaT cells were incubated with (*b, d*) or without (*a, c*) ginsenoside F1 (5 μm) for 24 h and then irradiated with 60 mJ per cm^2 UVB. (*d*) Phase-contrast microscopy 24 h after irradiation showed enhanced survival in ginsenoside-F1-treated cells.

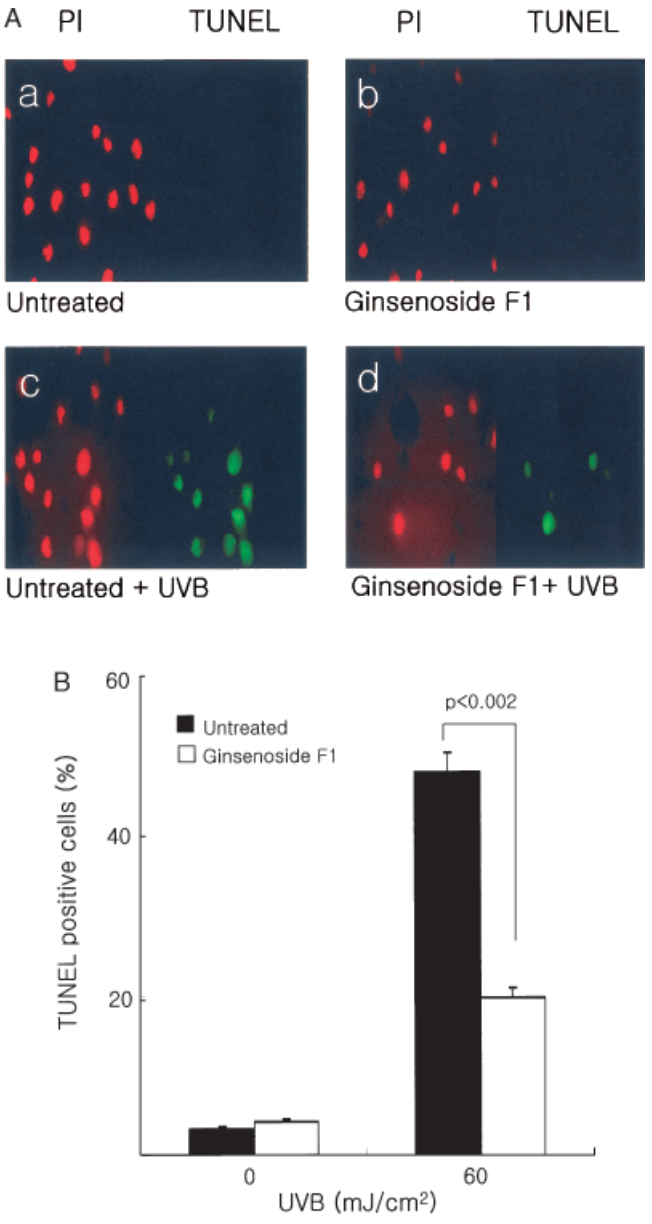


Figure 3. Ginsenoside F1 protects HaCaT cells from UVB-induced apoptosis. (*A*) HaCaT cells were incubated 24 h before UVB irradiation (60 mJ per cm^2) with or without ginsenoside F1 (5 μm). At 24 h after UVB irradiation, apoptotic nuclei or fragmented DNA were visualized using propidium iodide counterstaining or TUNEL staining. (*B*) About 200 cells were counted and labeled nuclei were expressed as a percentage of the total number of nuclei. Results are expressed as the mean ± SD of three experiments. Student's test was used for comparison of the means. (*C*) DNA was extracted from ginsenoside-F1-treated or untreated cells with or without UVB irradiation. DNA was electrophoresed on 2% agarose gel.

nucleotides 5′-CAACTACATGGTTTACATGTTCC-3′ (sense, bases 174–194 of human GAPDH cDNA) and 5′-GGACTGTGGTCATGAGTCCT-3′ (antisense, bases 570–589 of human GAPDH cDNA) were used (Suschek *et al*, 1999). For semiquantitative analysis, agarose gels were scanned by video documentation and the images were analyzed using the ImageMaster 2D Elite software (Amersham Biosciences Buckinghamshire, UK). Relative Bcl-2 and Bax mRNA expression was calculated by normalization to the expression of GAPDH.

Immunoblot analysis At 24 h after UVB irradiation (60 mJ per cm^2), ginsenoside-F1-treated or untreated cells were washed with PBS and lyzed on ice in RIPA buffer pH 8.5, as described elsewhere (Rodeck *et al*, 1997). Forty micrograms of total protein were analyzed under reducing conditions on 8% sodium dodecyl sulfate/polyacrylamide gels and blotted onto nitrocellulose membrane. The blot was incubated with an anti-Bax (0.5 μg per ml, Santa Cruz, Santa Cruz, CA), anti-Bcl-2 (2 μg per ml, Santa Cruz), anti-poly(ADP-ribose) polymerase (anti-PARP) (1 μg per ml, Santa Cruz), anti-Rb (2 μg per ml, Santa Cruz), anti-c-myb (2 mg per ml, Santa Cruz), anti-Brn-3a (2 μg per ml, Santa Cruz), and anti-Hsp 70 (0.5 μg per ml, Santa Cruz) polyclonal antibody. The reaction products were detected by chemiluminescence with the ECL kit (Amersham Biosciences) according to the manufacturer's instructions. Anti-Hsp 70 antibody was used to assess equal loading of the protein.

Plasmid constructs Plasmid constructs were carried out according to the method described by Smith *et al* (1998). Briefly, full-length human Brn-3a cDNA was amplified by PCR using primers 5′-GGATC CATGATGTCCATGAACAGCAAGCAGCC-3′ and 5′-GAATTCGTAAG TGGCAGAGAATTTCATCCG-3′ with human brain, whole Marathon-Ready cDNA (Clontech Palo Alto, CA) as template, and subcloned into the *Bam*HI and *Eco*RI sites of pcDNA4/TO/myc-His A (Invitrogen). The human Bcl-2 P2 promoter region (spanning between −746 and −8 bp relative to the ATG; Smith *et al*, 1998) were cloned upstream of the luciferase reporter gene in the pGL3 luciferase basic vector (Promega, Madison, WI). Correctness of all constructs was verified by sequencing.

Transient transfections Cells (1×10^5 cells) were transiently transfected with 10 μg of the promoter–reporter plasmid and 10 μg of the Brn-3a expression vector together with 2 mg of pCMV-β-galactosidase control vector using 10 mg of lipofectamine (Invitrogen) reagent, following the directions of the manufacturer. Transfected cells were treated with ginsenoside F1 and irradiated by UVB as described above. The efficiency of transfection of each sample was determined with a chemiluminescence assay for β-galactosidase activity using a commercial kit (Galactolight Plus, Tropics), and the values were subsequently used to equalize the values obtained from luciferase assays.

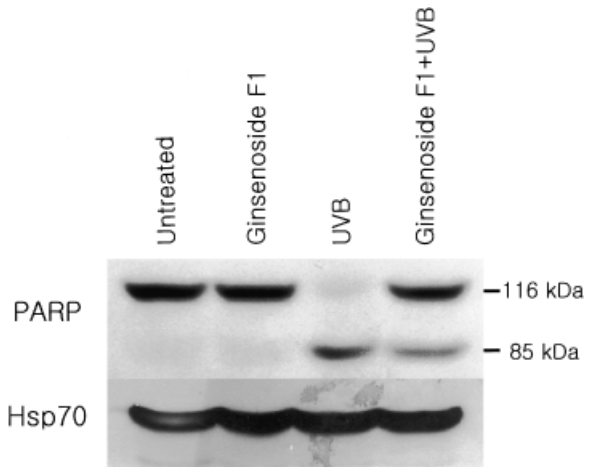


Figure 4. Ginsenoside F1 reduces UVB-induced PARP cleavage. HaCaT cells were incubated 24 h before UVB irradiation (60 mJ per cm^2) with or without ginsenoside F1 (5 μm). PARP cleavage was determined by immunoblot analysis 24 h after radiation. Equal loading of protein lysates was confirmed by anti-Hsp 70 antibody.

Luciferase assays Assays of luciferase activity were carried out using a commercially available kit (Promega, Madison, WI) and a Turner luminometer.

RESULTS

Ginsenoside F1 does not affect the vital parameters of HaCaT cells up to a concentration of 10 μm In order to understand the effects of ginsenoside F1 on HaCaT cells, we evaluated a possible alteration of vital parameters. Increasing doses of ginsenoside F1 (1–10 μm) did not cause HaCaT cell cytotoxicity as shown by Trypan blue dye exclusion and MTT (**Table I**). A concentration of 50 μm of ginsenoside F1 displayed a little cytotoxic effect in HaCaT keratinocytes.

Ginsenoside F1 protects HaCat cells from UVB-induced apoptosis In order to study the effects of ginsenoside F1 in UVB-induced HaCaT cell apoptosis, we used different doses of ginsenoside F1 in HaCaT cell cultures before the irradiation with different doses of UVB. As expected, UVB radiation drastically reduced viability of HaCaT cells in a dose-dependent manner. On the other hand, we observed a significant increase of viable cells in ginsenoside-F1-treated cells compared with untreated cells (**Fig 2*A***). Indeed, the treatment with 5 mM ginsenoside F1 led to maximal protective effects against UVB-induced cell death. At a UVB dose of 120 mJ per cm^2, however, ginsenoside-F1-treated cells showed a higher susceptibility to apoptosis than did untreated cells. UVB irradiation induced distinct morphologic alterations in HaCaT cells, which became round and detached from the plate (**Fig 2*B, c***), whereas the treatment with ginsenoside F1 prevented this alteration, as shown by the morphology of the cells (**Fig 2*B, d***).

To further investigate the protective effect of ginsenoside F1 against UVB-induced apoptotic cell death, TUNEL staining and DNA fragmentation assay were performed at 24 h after UVB irradiation using the ginsenoside-F1-treated or untreated cells. As shown in **Fig 3**, apoptotic nuclei and DNA strand breakages were clearly observed in untreated HaCaT cells at 24 h following 60 mJ per cm^2 UVB irradiation. The DNA cleavage, however, was reduced in ginsenoside-F1-treated cells.

During the UVB-induced apoptosis, caspase-3 is activated and cleaves several other apoptotic proteins such as PARP. We also examined apoptotic cleavage of the caspase-3 substrate PARP to its 85 kDa form by immunoblot analysis following 60 mJ per cm^2 UVB irradiation of HaCaT cells. Cleavage of PARP, as shown by the 85 kDa band, was observed at 24 h post-irradiation in untreated cells (**Fig 4**). In contrast, ginsenoside F1 markedly diminished the generation of the 85 kDa PARP fragment in HaCaT cells.

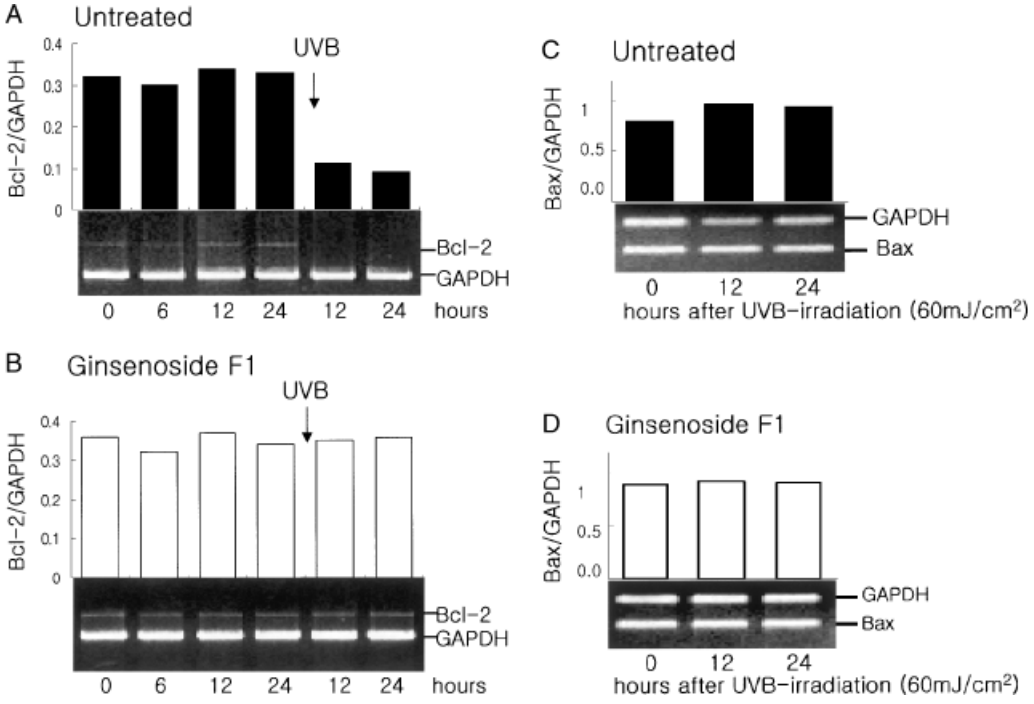


Figure 5. Effects of ginsenoside F1 and UVB on Bcl-2 and Bax mRNA expression. Cells were treated with (*B, D*) or without (*A, C*) ginsenoside F1 (5 μm) and harvested at different time-points prior to UVB radiation or post-UVB radiation (60 mJ per cm^2). Using quantitative RT-PCR, we examined the effects of ginsenoside F1 and UVB on the Bcl-2 and Bax mRNA expression of HaCaT cells, as described in *Materials and Methods*.

Taken together, these results demonstrate that ginsenoside F1 exerts an effective protection from UVB-induced apoptosis in HaCaT cells.

Ginsenoside F1 prevents UVB-induced downregulation of Bcl-2 in HaCaT cells In search of the molecular mechanism(s) responsible for the protective effects, we examined the mRNA expression and the relative amounts of the two proteins Bax and Bcl-2 in UVB-irradiated HaCaT cells with or without treatment with ginsenoside F1.

Fig. 5 shows the effects of ginsenoside F1 or UVB irradiation on Bcl-2 and Bax mRNA expression. GAPDH is used as a housekeeper gene to normalize the Bcl-2 and Bax genes. No difference in Bcl-2 mRNA expression was detected between ginsenoside-F1-treated and untreated cells in the absence of UVB irradiation. At 12 h after UVB irradiation, Bcl-2 mRNA expression levels were almost abolished in untreated HaCaT cells (**Fig 5*A***). On the other hand, Bcl-2 levels in ginsenoside-F1-treated cells were not downregulated by UVB irradiation (**Fig 5*B***), compared with those in unirradiated cells. The expression of Bax mRNA was not significantly changed after UVB irradiation with or without treatment with ginsenoside F1 (**Fig 5*C, D***).

The pattern of protein expression of Bcl-2 and Bax was similar to that of their mRNA expression (**Fig 6**).

Ginsenoside F1 counteracts the UVB-induced downregulated Brn-3a expression in HaCaT cells Although it is widely accepted that UVB irradiation decreases the level of Bcl-2 expression, the exact mechanism by which UVB downregulates the expression of Bcl-2 remains unclear. To understand the molecular mechanism for the inhibitory effect of ginsenoside F1 on UVB-induced downregulation of Bcl-2, we examined the expression patterns of various transcription factors that had been shown to regulate transcription of Bcl-2. c-myb has been shown to induce Bcl-2 promoter activity in murine T cells (Taylor *et al*, 1996) and Rb has been reported to activate transcription of Bcl-2 in epithelial cells by interacting with the activator protein 2 transcription factor (Decary *et al*, 2002). Brn-3a interacts with the P2 promoter of Bcl-2 gene and activates endogenous Bcl-2 expression in neuronal cells (Smith *et al*, 1998).

At 24 h after UVB radiation, protein extracts from the ginsenoside-F1-treated or untreated HaCaT cells were subjected to immunoblot with antibodies raised to c-myb, Rb, and Brn-3a. No difference in c-myb or Rb protein expression was detected between ginsenoside-F1-treated and untreated cells following UVB irradiation (**Fig 7**).

The level of Brn-3a protein expression was reduced by UVB irradiation in HaCaT cells. Interestingly, the UVB-induced reduction of Brn-3a expression was dramatically restored upon treatment with ginsenoside F1 (**Fig 7**), suggesting that Brn-3a transcription factor may be involved in the antiapoptotic effect of ginsenoside F1 through the induction of Bcl-2.

Overexpression of Brn-3a overcomes the repression of Bcl-2 promoter activity caused by UVB irradiation To establish a correlation between Brn-3a and Bcl-2 expression in UVB-irradiated HaCaT cells, we performed transient transfection assays. Bcl-2 P2 luciferase reporter construct contains the 738 bp of regulatory sequence immediately upstream of the Bcl-2 P2 promoter region (spanning –746 to –8 bp), which has been shown to be responsive to Brn-3a transcription factor (Smith *et al*, 1998). HaCaT cells were transfected with the Bcl-2 P2 luciferase reporter constructs following the treatment with ginsenoside F1 and UVB irradiation. Whereas UVB irradiation resulted in a 4-fold decrease in luciferase activity in untreated HaCaT cells, no change was observed in luciferase activity in ginsenoside-F1-treated cells, compared with unirradiated cells (**Fig 8**). To determine if the overexpression of Brn-3a overcomes the repression of luciferase activity under Bcl-2 promoter control

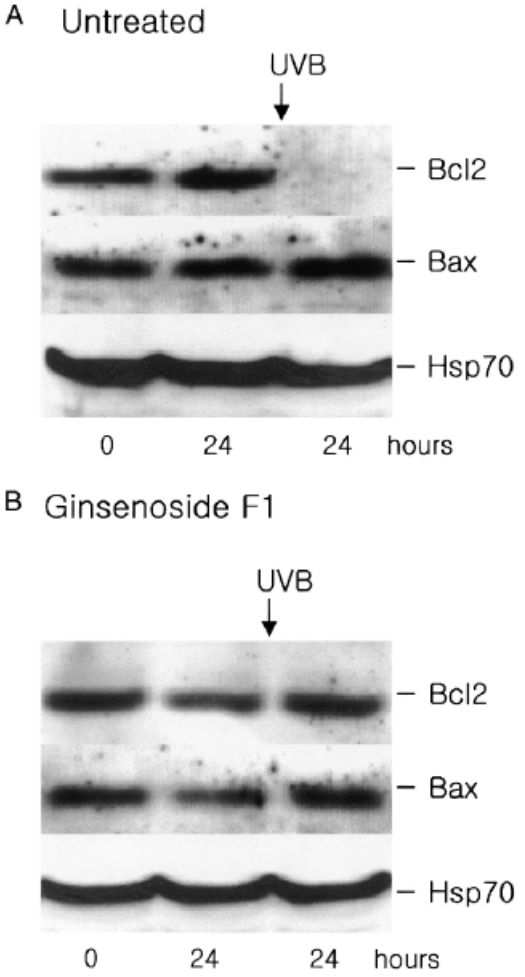


Figure 6. Effects of ginsenoside F1 and UVB on Bcl-2 and Bax protein expression. Cells were treated with (*B*) or without (*A*) ginsenoside F1 (5 μm) and harvested at different time-points prior to UVB radiation or post-UVB radiation (60 mJ per cm^2). The expression of Bcl-2 and Bax protein was examined by immunoblots as described in *Materials and Methods*. Equal loading of protein lysates was confirmed by anti-Hsp 70 antibody.

caused by UVB irradiation, HaCaT cells were cotransfected with the Bcl-2 P2 luciferase reporter construct and the pBrn-3a expression vector or empty expression vector followed by exposure to UVB. Cotransfection of HaCaT cells with the Brn-3a expression vector restored the decrease of luciferase activity caused by UVB irradiation (**Fig 8**). These data suggest that UVB irradiation may downregulate Bcl-2 expression via a downregulation of Brn-3a transcription factor in HaCaT cells, and ginsenoside F1 may overcome the repression of Bcl-2 promoter activity via upregulation of Brn-3a expression in UVB-irradiated cells.

DISCUSSION

Ginseng is a highly valued herb in the Far East and has gained popularity in the West during the last decade. The major active components of ginseng are ginsenosides, a diverse group of steroidal saponins, which demonstrate the ability to target a myriad of tissues, producing an array of pharmacologic responses (Gillis, 1997). Many mechanisms of ginsenoside activities still remain unknown, however.

In this study we first demonstrate that ginsenoside F1 protects human HaCaT cells from UVB-induced damage *in vitro*. In particular, ginsenoside F1 effectively reduced cell death and apoptotic

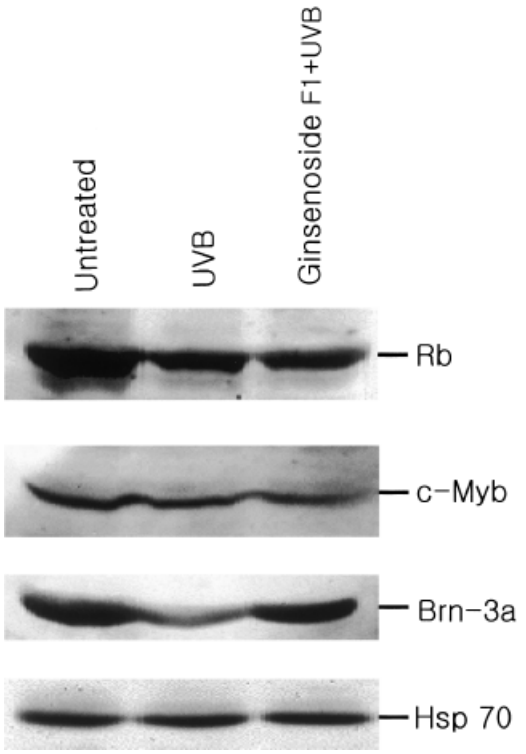


Figure 7. Effects of ginsenoside F1 and UVB on Rb, c-myb, and Brn-3a expression. HaCaT cells were incubated 24 h before UVB irradiation (60 mJ per cm^2) with or without ginsenoside F1 (5 μm). At 24 h after UVB exposure, attached and nonattached cells were harvested and analyzed by immunoblotting using anti-Rb, anti-c-myb, and anti-Brn-3a antibodies as described in *Materials and Methods.* Anti-Hsp 70 antibody was used to assess equal loading of the protein.

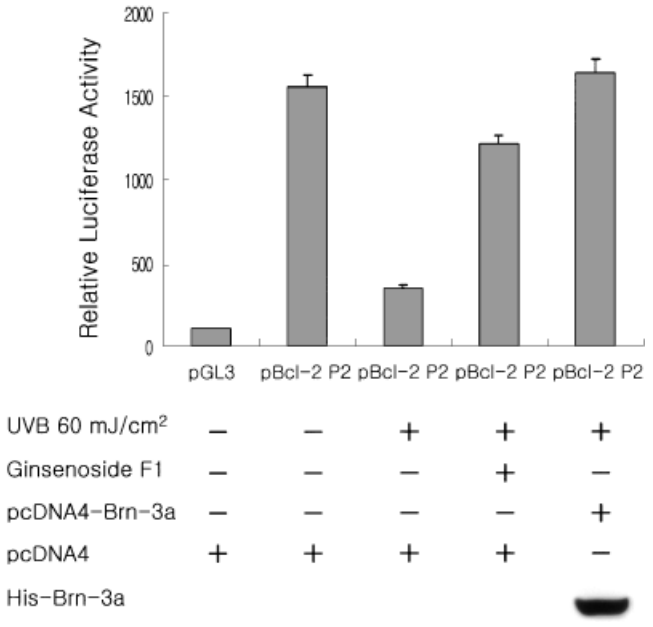


Figure 8. Assay of luciferase activity of the Bcl-2 P2 promoter in HaCaT cells. Cells were transfected with 10 μg of Bcl-2 P2 luciferase reporter plasmid in the presence of 10 μg of the Brn-3a expression vector or the empty expression vector together with 2 mg of pCMV-β-galactosidase control vector. After 3 h of transfection, cells were treated with or without ginsenoside F1 (5 μm) for 24 h and then exposed to UVB at 60 mJ per cm^2. The luciferase activity is plotted relative to the promoterless pGL3 basic vector, which was assigned a value of 100. Results are expressed as the mean ± SD of three independent experiments, each analyzed in duplicate. Overexpressed Brn-3a was detected by immunoblot with His antibody.

DNA cleavage after UVB radiation. In addition, we show that Bcl-2 mRNA expression was not affected by UVB in ginsenoside-F1-treated HaCaT cells, whereas it was markedly downregulated in untreated cells. Thus, it is conceivable that ginsenoside F1 protects human HaCaT cells from UVB-induced apoptosis by maintaining constant levels of Bcl-2. Furthermore, this positive regulation of Bcl-2 might be mediated by Brn-3a transcription factors (Pit-1, Oct-1 and Oct-2, unc-86) binding domain (POU) domain transcription factor in ginsenoside-F1-treated HaCaT cells.

Brn-3a, a member of the Brn-3 family of type IV POU domain transcription factors, protects sensory neurons from apoptotic programmed cell death (Smith *et al*, 1997). The antiapoptotic effect of Brn-3a is mediated via the upregulation of Bcl-2 expression. Brn-3a interacts with the P2 promoter of Bcl-2 gene and activates endogenous Bcl-2 expression in neuronal cells (Smith *et al*, 1998). Our experiments showed that UVB irradiation caused a decrease in Bcl-2 and Brn-3a expression in HaCaT cells, but not in ginsenoside-F1-treated cells (**Figs 6, 7**). Furthermore, overexpression of Brn-3a overcame the repression of Bcl-2 promoter activity caused by UVB irradiation (**Fig 8**). These data suggest that the positive regulation of Bcl-2 gene by Brn-3a provides a possible pathway by which ginsenoside F1 is able to confer HaCaT cells with resistance to UVB-induced apoptosis. Further experiments are needed to precisely define the molecular mechanism by which ginsenoside F1 affects the expression of Bcl-2. We also have to test whether HaCaT cells are resistant to UVB-induced apoptosis when the cells are stably transfected with Brn-3a.

UVB radiation activates caspase-3, which cleaves several other apoptotic proteins such as PARP (Mellroy *et al*, 1999). During the apoptosis, PARP is cleaved from the 116 kDa intact form into the 85 kDa form. Recently, it has been reported that Bcl-2 can protect cells from apoptosis by acting at a point downstream from release of mitochondrial cytochrome c, thereby preventing a caspase-3-dependent proteolytic cascade (Swanton *et al*, 1999). In our study, treatment of ginsenoside F1 inhibited UV-induced PARP cleavage in HaCaT cells, suggesting that this molecule may prevent caspase activation through a Bcl-2-mediated antiapoptotic pathway.

The death-suppressing activity of Bcl-2 is regulated by Bax, which promotes cell death. The ratio of these two proteins is considered to be important when determining whether the cell undergoes apoptosis after an appropriate stimulus (Yang and Korsmeyer, 1996). Previously, it has been shown that Bcl-2 expression is reduced after UVB irradiation in rat and mouse skin (Gillardon *et al*, 1994; Li *et al*, 1996), but, in contrast, the expression of Bax is not changed (Gillardon *et al*, 1994). Our experiments also showed that neither UVB nor ginsenoside F1 had any influence on Bax mRNA expression.

In conclusion, these data demonstrate that ginsenoside F1 protects HaCaT keratinocytes from UVB-induced apoptosis, at least in part through the Brn-3a-mediated transcriptional regulation of Bcl-2. Thus, these results suggest a new rationale for use of ginsenoside F1 to prevent UVB-induced skin damage.

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