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Ginsenosdie F1과 EGCG의 상승작용에 의한 자외선조사에 의한 세포 사멸 방지

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Combining Ginsenoside F1 with (-)-Epigallocatechin Gallate Synergistically Protects Human HaCaT Keratinocytes from Ultraviolet B-Induced Apoptosis

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요 약: 피부는 외부에 노출되어 있기 때문에 외부로부터의 자극에 의하여 손상받기 쉽다. 그러한 외부적 자극 중 자외선은 피부노화의 주요한 원인으로 손꼽힌다. 자외선 중 특히 280~320 nm의 파장을 갖는 UVB (ultraviolet-B)는 피부노화의 가장 중요한 요인으로서 피부화상이나 피부암을 유발한다. 고선량의 자외선에 노출된 세포는 복구할 수 없는 심각한 DNA 손상을 입게 되는데, 이 경우 세포사멸(apoptosis) 현상은 이러한 세포들의 죽음을 유도하여 이들이 종양으로 발전하는 것을 막는다. 따라서, 세포 손상 정도에 따라 특이적으로 세포사멸을 유도하거나 막는 것이 암 발생을 억제하면서 세포의 항상성을 유지하는데 매우 중요하다. 이러한 세포 사멸을 조절하는데 가장 중요한 단백질로 알려진 것이 Bcl-2이다. Bcl-2는 피부세포에서 자외선 조사에 의해 그 발현이 급격히 감소되며 이를 통해 피부세포가 사멸한다. 따라서, 자외선 조사시에 Bcl-2의 발현감소를 막을 수 있다면 피부세포의 사멸을 막을 수 있고, 이를 통해 자외선에 의한 피부손상을 방지하여 노화를 억제할 수 있을 것으로 기대한다. 본 연구에서는 인삼의 진세노사이드 F1(20-O-β-D-글루코피라노실-20(S)-프로토파낙사트리올)과 녹차의 주요 효능 성분인 EGCG ((-) 에피갈로카테킨-3-갈레이트)을 함유하는 조성물이 자외선조사에 의한 피부세포사멸을 억제하여 그 손상을 방지하는 데 탁월한 효과가 있음을 밝힌 것이다. 즉, 진세노사이드 F1과 EGCG을 단독으로 처리했을 때에는 효과가 없는 낮은 농도의 두 화합물을 동시에 처리하게 되면, 상승작용을 통해 자외선 조사시 유발되는 Bcl-2의 발현 감소 및 그의 전사인자인 Brn-3a의 발현 감소를 억제함과 동시에, Rb 단백질의 탈인산화를 저해함으로써 자외선에 의한 세포사멸을 방지하였다. 본 연구결과를 통해 낮은 농도의 진세노사이드 F1과 EGCG를 동시에 처리함으로써 자외선 노출에 의한 세포손상을 방지하여 피부 노화를 억제할 수 있는 물질로서 활용할 수 있다는 가능성을 제시하였을 뿐만 아니라, 단가가 높은 두 화합물을 낮은 농도(단독 사용시 각각 2.5배, 5배 농도 필요)로 사용함으로써 원료비를 낮추어 고기능성 제품을 보다 저렴한 가격에 공급할 수 있는 기회를 제공할 수 있을 것으로 기대한다.

Abstract: Ginsenosides and green tea extracts show a variety of biomedical efficacies such as anti-aging, anti-oxidation and anti-tumor-promotion effects. (-)-Epigallocatechin-3-gallate (EGCG) has been reported to inhibit the UVB-induced apoptosis by increasing the Bcl-2-to-Bax ratio. We have previously shown that ginsenoside F1 protects human HaCaT cells from ultraviolet-B (UVB)-induced apoptosis by maintaining constant levels of Bcl-2 and Brn-3a. Here, we investigate the combined effect of ginsenoside F1 and EGCG on the protection of human HaCaT keratinocyte against UVB-induced apoptosis. When treated individually, although 5 μM ginsenoside F1 and 50 μM EGCG protected cells from UVB-induced apoptosis, 2 μM ginsenoside F1 or 10 μM EGCG treatment showed very little protection effect. However, cotreatement of 2 μM ginsenoside F1 and 10 μM EGCG successfully protected HaCaT cells from UVB-induced cell death. As expected, combining ginsenoside F1 and EGCG efficiently prevented UVB-induced decrease of Bcl-2 and Brn-3a expression. In addition, cotreatment with ginsenoside F1 and EGCG prevented the dephosphorylation of Rb, whereas individual treatment with ginsenoside F1 or EGCG failed to prevent the dephosphorylation of Rb even at high concentrations.

Keywords: *ginsenoside F1, Bcl-2, EGCG, Rb, Brn-3a*

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1. Introduction

Ultraviolet radiation (UV), in particular ultraviolet B (UVB) with a wavelength range between 290 and 320 nm, is the major environmental cause of skin damage[1]. Exposure of cells to UVB radiation results in the loss of keratinocyte viability, an increase in membrane blebbing[2], cytoskeletal molecular changes[3], and apoptosis[4].

(-)-Epigallocatechin gallate (EGCG) is the most abundant polyphenolic catechin isolated from green tea (Figure 1A). EGCG are known to be effective free radical scavengers, potent antioxidants[5] and cancer-preventive agent. It has been also shown that EGCG protects against UVB-induced skin carcinogenesis in mice[6] and inhibits UVB-induced inflammatory responses and photocarcinogenesis in human skin[7]. Furthermore, a recent report shows that EGCG inhibits the UV-induced apoptosis in normal epidermal keratinocytes by increasing the Bcl-2-to-Bax ratio, but not in epidermoid carcinoma cells[8].

Ginsenosides represent the major active ingredients of ginseng, which show a variety of biomedical efficacies, such as immune modulation, anti-aging, anti-inflammation, and anti-oxidation in the CNS, cardiovascular, endocrine, and immune systems[9-11]. Ginsenoside F1 (20-O--D-glucopyranosyl-20 (S)-protopanaxatriol) is an enzymatically modified derivative of ginsenoside Rg1 (Figure 1B). In our previous study, we showed that UVB irradiation downregulates Bcl-2 expression via a downregulation of Brn-3a transcription factor in HaCaT cells and ginsenoside F1 protected these cells against UVB-induced apoptosis by maintaining constant levels of Brn-3a and the corresponding inhibition of Bcl-2 downregulation[12].

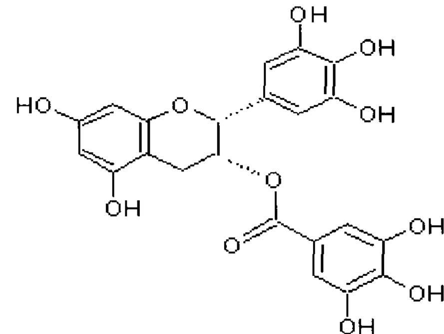
In this study, we investigated the possible synergistic or additive effects of cotreatment with ginsenoside F1 and EGCG on anti-apoptotic activity. We present the first evidence that cotreatment with ginsenoside F1 and EGCG synergistically inhibits the UVB-induced apoptosis of HaCaT cells by maintaining constant level of Bcl-2, Brn-3a expressions and Rb phosphorylation state.

2. Materials and Methods

2.1. Chemicals

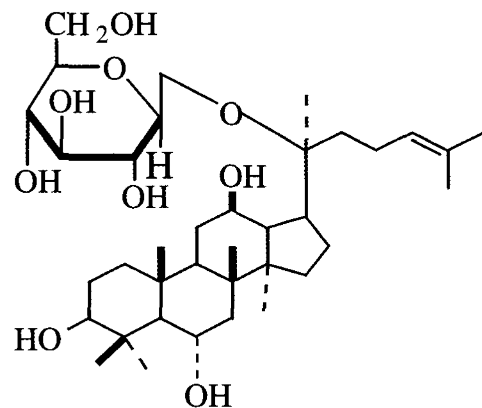
Ginsenoside F1 was prepared as described previously

(-)-Epigallocatechin gallate



(A)

Ginsenoside F1



(B)

Figure 1. Structure of ginsenoside F1 and EGCG.

[12]. Ginsenoside F1 was dissolved in 100% Ethanol. EGCG was purchased from Sigma (St. Louis, MO) and dissolved in DMSO. The final concentration of either ethanol or DMSO was kept in below 0.1% (v/v) in all the cell cultures and did not exert any detectable changes in cell growth or apoptosis.

2.2. Cell Culture and Treatment

Human HaCaT keratinocytes were obtained from Dr Fusenig of the German Cancer Research Center (DKFZ) and the cells (1.5-2×10^5 cells per well) placed in 6-well plates were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum for 24 h. After serum-starvation for 24 h, HaCaT cells were treated with either ginsenoside F1 (0, 2, 5, 10, 20 and 50 μM) or EGCG (0, 5, 10, 20, 50 and 100 μM) alone, or were co-treated with ginseno-

side F1 (2 μM) and EGCG (10 μM) and cells were incubated for 24 h prior to irradiation with UVB.

2.3. UVB Irradiation

HaCaT cells were rinsed twice with PBS and exposed to UVB at 60 mJ per cm^2, whose intensity was monitored with a IL-1700 radiometer. UVB radiation source was provided by a bank of Sankyo Denki G15T8E, a fluorescent bulb emitting 270~320 nm wave-length 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 each compound at various concentrations either alone or in combination and followed up to 24 h.

2.4. 3-(4,5-dimethylthiazol-2-yl)-2,5 Diphenyl-tetrazolium bromide (MTT) Assay

Cell viability was determined by the MTT assay as described by Fumelli *et al.* (13). After each agent or UVB treatment, the cells were cultured for 24 h. The cells were washed with PBS, 200 μL of MTT (0.05 mg/mL) was added to each well, and the cells were incubated for 4 h at 37℃. The supernatant was then removed, 200 μL DMSO was added to each well to dissolve the formazan product. The results of MTT assays were expressed as optical density units (OD) using an ELISA reader (Thermo Max, Molecular Devices Co., Sunnyvale, CA, USA) at 540 nm. The ratio of viable cells was expressed as a percentage of the OD value obtained without UVB treatment.

2.5. Assessment of Apoptosis by Annexin V

Apoptotic cell death was measured using annexin V-FITC conjugate[14]. Cells were incubated in serum-free DMEM containing 0.5 mg/mL annexin V-FITC and 1 mg/mL PI for 15 min. The fluorescence of annexin V-FITC and PI were monitored by COULTER EPICS XL-MCLATM flow cytometry.

2.6. Immunoblot Analysis

At 24 h after UVB irradiation (60 mJ per cm^2), HaCaT cells were washed with PBS and lysed on ice in RIPA buffer pH 8.5, as a method described elsewhere[15]. Forty micrograms of total protein were analyzed under reducing conditions on 8% or 4~15% sodium dodecyl sulfate/polyacrylamide gels and blotted on to nitrocellulose membrane. The blot was incubated with an anti-Bcl-2 (2 μg/mL, Santa Cruz, Santa Cruz, CA), anti-PARP (1 μg/mL, Santa Cruz, Santa Cruz, CA), anti-Brn-3a (2 μg/mL, Santa Cruz, Santa Cruz, CA), anti-Rb (2 μg/mL, Santa Cruz, Santa Cruz, CA), and anti-Hsp 70 (0.5 μg/mL, Santa Cruz, Santa Cruz, CA) polyclonal antibody. The reaction products were detected by chemiluminescence with the ECL kit (Amersham BioSciences) according to the manufacturer's instructions.

Table 1. Effect of Either Ginsenoside F1 or EGCG on the Viability and Cytotoxicity of HaCaT Cellsa

Ginsenoside F1	MTT OD±SD[b]	EGCG	MTT OD±SD[b]
Control	1.229±0.03	Control	1.198±0.02
2 μM	1.209±0.10	5 μM	1.201±0.05
5 μM	1.242±0.09	10 μM	1.215±0.03
10 μM	1.210±0.05	20 μM	1.219±0.02
20 μM	1.225±0.06	50 μM	1.452±0.10
50 μM	0.704±0.10	100 μM	0.504±0.10

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

3. Results

3.1. Viability of HaCaT Cells after Treatment with Either Ginsenoside F1 or EGCG

The effects of EGCG on growth of HaCaT cells were assessed by MTT assay. Treatment of unirradiated HaCaT cells with EGCG for 24 h had no effect on their proliferation up to 20 μM; however, treatment with 50 μM EGCG slightly increased the proliferation of HaCaT cells (Table 1). The proliferation of HaCaT cells treated with 100 μM EGCG was significantly inhibited. As reported previously[12], ginsenoside F1 did not affect the vital parameters of HaCaT cells up to a concentration 20 μM, but treatment with 50 μM ginsenoside F1 displayed the cytotoxic effect in cells.

3.2. Effect of Combination Treatment with Ginsenoside F1 and EGCG on Protection of HaCaT Cells Against UVB-induced Apoptosis

The effects of ginsenoside F1 and EGCG, either alone or in combination, on the UVB-induced apoptosis

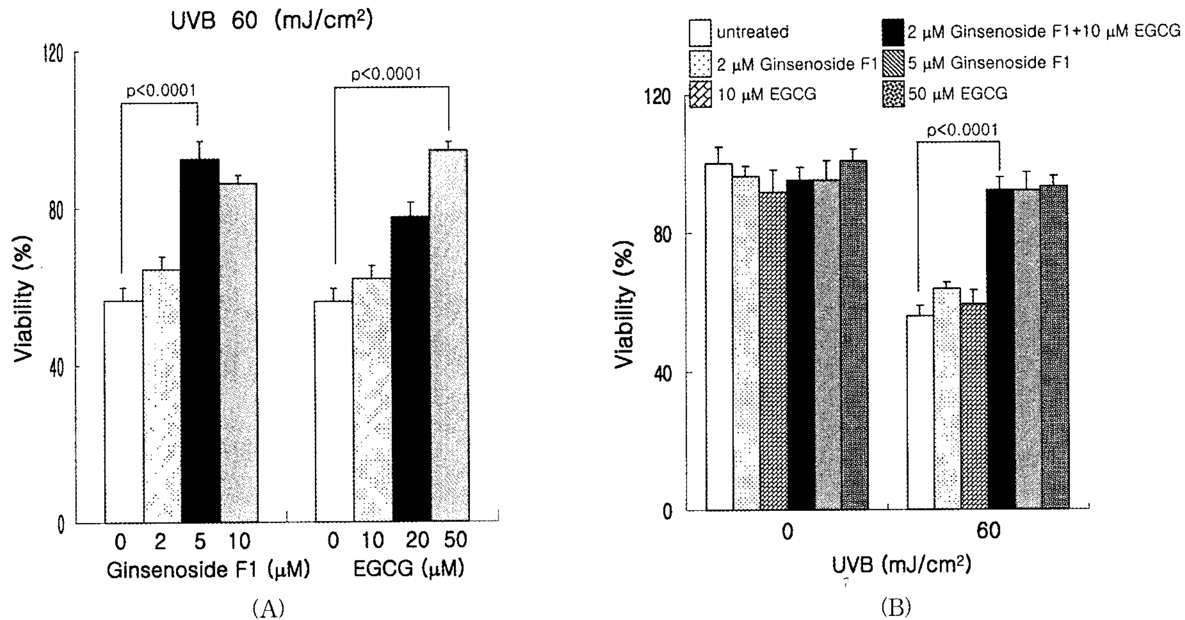


Figure 2. Effect of combination treatment with ginsenoside F1 and EGCG on prevention of UVB-induced apoptosis. (A) HaCaT cells were treated for 24 h with different doses of either ginsenoside F1 (0, 2, 5, and 10 μM) or EGCG (0, 10, 20, and 50 μM) alone and were exposed to 60 mJ/cm^2 UVB. At 24 h after 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 treated with ginsenosdie F1, EGCG, and in combination for 24 h and then irradiated with 60 mJ/cm^2 UVB. The data are presented as means ∓ SD from five repeated experiments.

of HaCaT cells were assessed by the MTT assay (Figure 2). As shown previously, irradiation with 60 mJ per cm^2 UVB induced cell death of HaCaT. The pretreatment with either ginsenoside F1 or EGCG alone for 24 h before UVB irradiation inhibited the UVB-induced cell death in a dose-dependent manner. As shown in Figure 2A, individual treatment with 2 μM ginsenoside F1 or 10 μM EGCG did not significantly increase the viability of UVB-irradiated cells as estimated by MTT assay. However, interestingly, cotreatment with 2 μM ginsenoside F1 and 10 μM EGCG effectively reduced cell death induced by UVB irradiation (Figure 2B). The synergy between ginsenoside F1 and EGCG in apoptosis prevention was further confirmed by flow cytometry analysis. The necrotic cells lost cell membrane integrity that permits PI entry. Viable cells exhibit Annexin V-/PI-; early apoptotic cells exhibit Annexin V+/PI-; late apoptotic cells or necrotic cells exhibit Annexin V+/PI+. At 24 h after UVB irradiation, the apoptotic cells (Annexin V+/PI-) had clearly increased compared to the unirradiated cells (Table 2). The pretreatment of 2 μM ginsenoside F1 and 10 μM EGCG in combination resulted in a decrease in Annexin V+/ PI- cells, which is visible by a right shift of the cell population presented in Figure 3E. However, single-agent treatment with either 2 μM ginsenoside F1 or 10 μM EGCG had no such effect (Figure 3C and D).

PARP is a specific substrate for caspase, which play a crucial role in the execution stage of apoptosis. In this study, we showed that the synergy between ginsenoside F1 and EGCG inhibited caspase activation induced by UVB irradiation. Indeed, the 85 kDa fragment, representing the cleaved form of PARP, was clearly less produced by treating the two agents together (2 μM ginsenoside F1 and 10 μM EGCG), compared with that produced by treating either single agent (Figure 4).

3.3. Effect of Combination Treatment with Ginsenoside F1 and EGCG on Inhibition of UVB-induced Decrease of Bcl-2 in HaCaT Cells

To determine whether the protective effect of cotreatment with ginsenoside F1 and EGCG against UVB-

Table 2. Effect of Ginsenoside F1 and EGCG on Preventionof UVB-Induced Apoptosis

Treatment	Annexin V^+/PI^- cells (%)	Annexin V^+/PI^+ cells (%)
Control	7.5±0.4	5.4±0.7
Control+UVB 60 mJ/cm^2	55.1±0.9	11.2±1.6[a]
2 μM ginsenoside F1+UVB 60 mJ/cm^2	52.0±1.2	9.3±1.1
10 μM EGCG+UVB 60 mJ/cm^2	50.4±1.4	12.1±0.8
2 μM ginsenoside F1+10 μM EGCG+ UVB 60 mJ/cm^2	9.3±0.7	6.7±1.0
5 μM ginsenoside F1+UVB 60 mJ/cm^2	9.6±1.6[a]	6.2±0.6
50 μM EGCG+UVB 60 mJ/cm^2	8.1±0.8	7.1±1.2

HaCaT cells were treated with the indicated concentration of ginsenosdie F1, EGCG, and in combination for 24 h and then irradiated with 60 mJ/cm^2 UVB. At 24 h after UVB irradiation, cells were incubated with annexin V-FITC and PI. The fluorescence of annexin V-FITC and PI were monitored by a flow cytometer. Each data represents the mean ∓ S.D. for three separate determinations.

[a]$p \leq 0.05$ considered significant.

induced apoptosis was associated with an enhanced effect on Bcl-2 expression, western blot analysis was performed on extracts from the cells treated with ginsenoside F1, EGCG, or in combination for 24 h following UVB irradiation.

As expected, at 24 h Bcl-2 protein levels were downregulated by UVB irradiation compared with unirradiated HaCaT cells (Figure 5). Individual treatment with 50 μM EGCG and 5 μM ginsenoside F1 successfully inhibited the downregulation of Bcl-2 protein induced by UVB irradiation, whereas individual treatment with 10 μM EGCG or 2 μM ginsenoside F1 did not inhibit it. However, cotreatment with 10 μM EGCG and 2 μM ginsenoside F1 dramatically inhibited the decrease of Bcl-2 protein expression in UVB irradiated HaCaT cells.

3.4. Effect of Combination Treatment with Ginsenoside F1 and EGCG on Restoring of UVB-induced Downregulation of Brn-3a in HaCaT Cells

In the previous study, we showed that ginsenoside F1 protects HaCaT keratinocytes from UVB-induced apoptosis, at least in part, through the Brn-3a-mediated transcriptional regulation of Bcl-2. To investigate whether cotreatment with ginsenoside F1 and EGCG protects HaCaT cells from UVB-induced apoptosis through the Brn-3a pathway, we examined the expression pattern of Brn-3a protein.

The level of Brn-3a protein expression was reduced by UVB irradiation in HaCaT cells. Cotreatment with 2 μM ginsenoside F1 and 10 μM EGCG dramatically restored the UVB-induced reduction of Brn-3a expression (Figure 6), but individual treatment with either 2 μM ginsenoside F1 or 10 μM EGCG did not, suggesting that synergy between ginsenoside F1 and EGCG

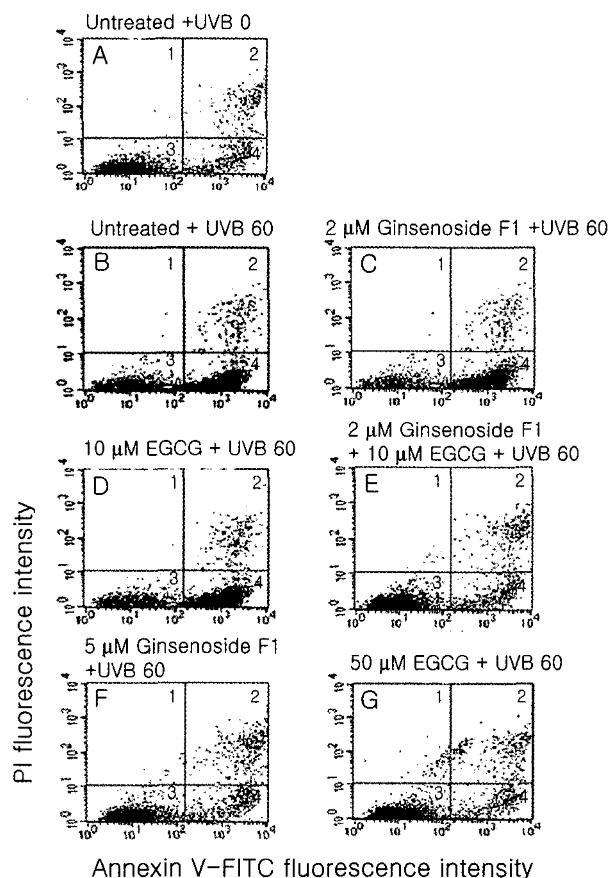


Figure 3. Flow cytometric analysis indicating a synergistic protective effect of ginsenoside F1 and EGCG against UVB-induced apoptosis. HaCaT cells were treated with ginsenoside F1 (2, 5 μM), ECGC (10, 50 μM), or in combination of 2 μM ginsenoside F1 and 10 μM EGCG for 24 h and were exposed to UVB (60 mJ/cm^2). At 24 h after UVB irradiation, cells were harvested and double staining with Annexin V-FITC and PI was performed as described in Material and Methods. Symbols:1, cells exhibit Annexin V-/PI+; 2, late apoptotic cells or necrotic cells exhibit Annexin V+/PI+; 3, viable cells exhibit Annexin V-/PI-; 4, early apoptotic cells exhibit Annexin V+/PI-.

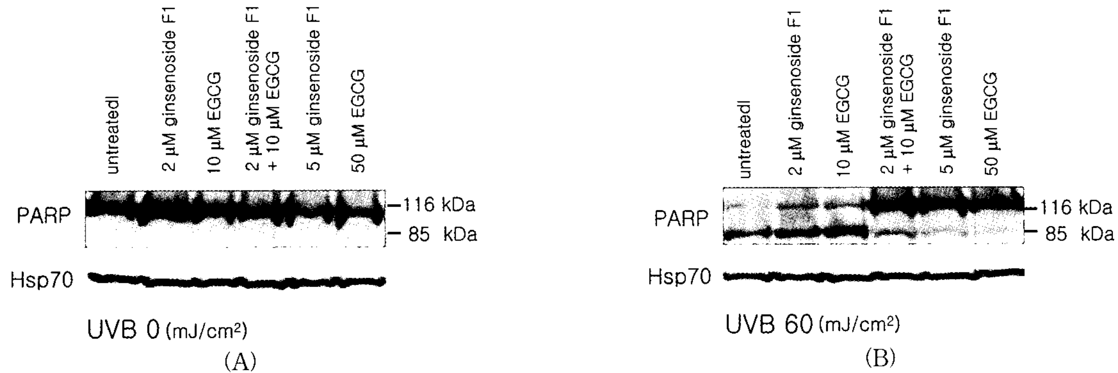


Figure 4. Reduction of UVB-induced PARP cleavage by cotreatment with ginsenoside F1 and EGCG. HaCaT cells were incubated 24 h before UVB irradiation (60 mJ/cm^2) with ginsenoside F1 (2, 5 μM) or ECGC (10, 50 μM), or in combination of 2 μM ginsenoside F1 and 10 μM EGCG. PARP cleavage was determined by immunoblot analysis at 24 h after irradiation. Equal loading of protein lysates was confirmed by Anti-Hsp 70 antibody.

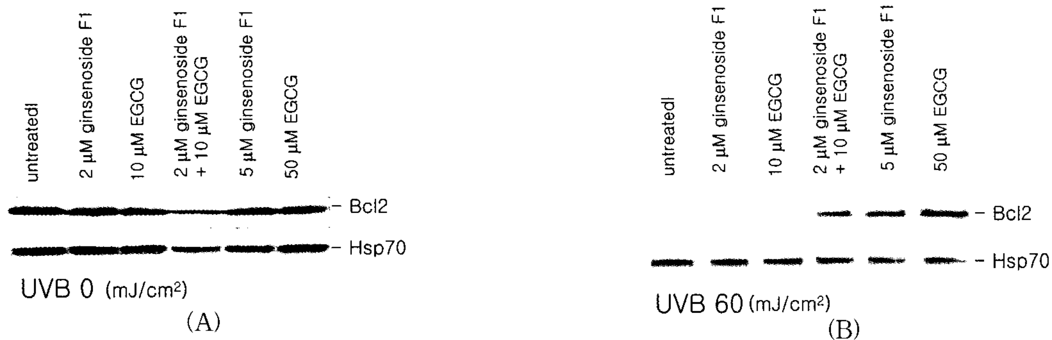


Figure 5. Positive effects of ginsenoside F1 and ECCG on Bcl-2 expression in UVB-irradiated cells. HaCaT cells were treated with ginsenoside F1 (2, 5 μM) or ECGC (10, 50 μM), or in combination of 2 μM ginsenoside F1 and 10 μM EGCG for 24 h and were either UVB (60 mJ/cm^2) (B) or sham-irradiated (A). The expression of Bcl-2 protein was examined by immunoblots exactly as descried under "Materials and Methods." Equal loading of protein lysates was confirmed by Anti-Hsp 70 antibody.

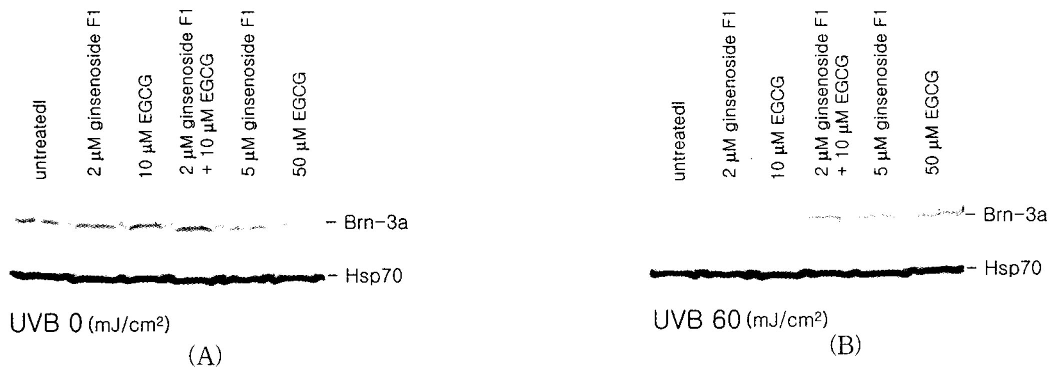


Figure 6. Positive effects of ginsenoside F1 and ECCG on Brn-3a expression in UVB-irradiated cells. HaCaT cells were treated with ginsenoside F1 (2, 5 μM) or ECGC (10, 50 μM), or in combination of 2 μM ginsenoside F1 and 10 μM EGCG for 24 h and were either UVB (60 mJ/cm^2) (B) or sham-irradiated (A). At 24 h after irradiation attached and nonattached cells were harvested and analyzed by immunoblotting using anti-Brn-3a antibodies as descried in "Materials and Methods". Anti-Hsp 70 antibody was used to assess equal loading of the protein.

in prevention of UVB-induced apoptosis is mediated by upregulation of Brn-3a protein in HaCaT cells.

3.5. Synergistic Inhibition of Dephosphorylation of Rb by Combination of Ginsenoside F1 and EGCG.

Rb has been reported to activate transcription of the *bcl-2* gene in epithelial cells and to inhibit apoptosis [16]. Also it has been reported that Rb was dephosphorylated and cleaved during apoptosis in most instances, the serine/threonine protein phosphatase inhibitors could prevent this dephosphorylation and subsequent apoptosis in certain cell types[17]. Therefore, it has been recognized that the dephosphorylation of Rb not only promotes cell cycle arrest and a return to the G1 phase, but also appears to be a key event in most instances of apoptosis. We have previously shown that no difference in Rb protein expression was detected between ginsenoside F1-treated and untreated cells following UVB irradiation. Rb was present in both hypophosphorylated and hyperphosphorylated forms in HaCaT cells without UVB exposure. Following UVB exposure, Rb became dephosphorylated and its hypophosphorylated form was accumulated. Surprisingly, Rb continued to be hyperphosphorylated by cotreatment with 2 μM ginsenoside F1 and 10 μM EGCG, but not by single-agent treatments with ginsenoside F1 or EGCG even at high concentrations (Figure 7).

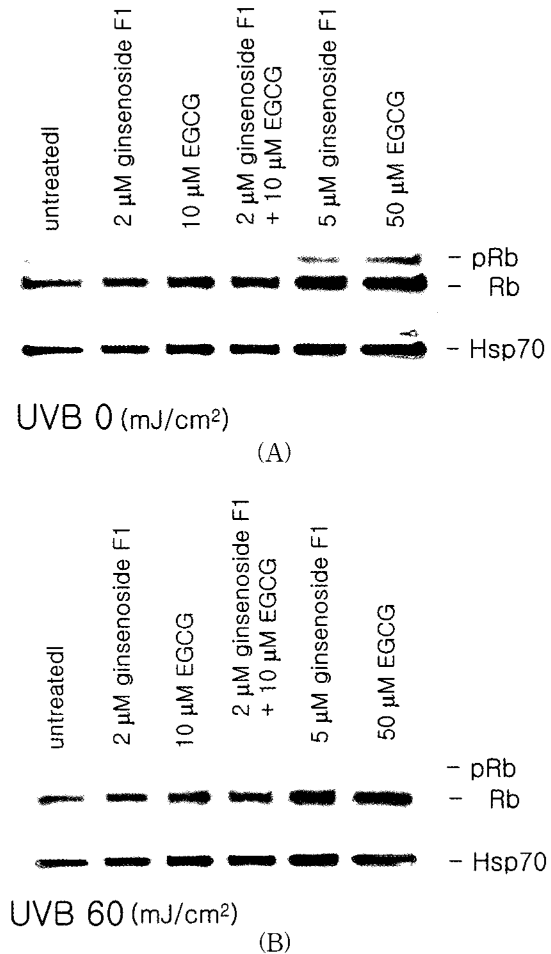


Figure 7. Synergistic effects of ginsenoside F1 and EGCG on phosphorylation of Rb in UVB-irradiated cells. HaCaT cells were treated with ginsenoside F1 (2, 5 μM) or ECGC (10, 50 μM), or in combination of 2 μM ginsenoside F1 and 10 μM EGCG for 24 h and were either UVB (60 mJ/cm^2) (B) or sham-irradiated (A). At 24 h after irradiation total Rb was detected by western blot and two major forms of the protein can be resolved: hypophosphorylated and phosphorylated (p) Rb.

4. Discussion

Ginsenosides and green tea extracts show the biological activities such as anticancer and antioxidative effects[9-11,18,19]. In human keratinocytes, ginsenoside F1 has been found to protect HaCaT cells from UVB-induced apoptosis by inhibiting the downregulation of *bcl-2* gene[12]. The major green tea polyphenol EGCG also promotes keratinocyte survival and inhibits the UV-induced apoptosis by increasing the Bcl-2 expression[8]. Despite the increasing interest in beneficial skin effects of gisenoside F1 or EGCG, it is not practical to use these compounds as photo-protectants against UVB-induced skin damage due to their rather high effective concentrations.

In this study, we first demonstrate that cotreatment with ginsenoside F1 and EGCG at low concentrations synergistically protects human HaCaT cells from UVB-induced damages. Although individual treatment of 5 μM ginsenoside F1 and 50 μM EGCG efficiently protected HaCaT cells from UVB-induced apoptosis, individual treatment with 2 μM ginsenoside F1 or 10 μM EGCG showed little protective effects. However, we found that the cotreatment with 2 μM ginsenoside F1 and 10 μM EGCG almost completely inhibited the UVB-induced apoptosis of HaCaT keratinocytes. Furthermore, the low concentration cotreatment with these two agents

produced a synergistic effect on the restoring of the UVB-induced decrease of Bcl-2 and Brn-3a expressions. In our previous study, ginsenoside F1 was found to protect the HaCaT cells from the UVB-induced damage by maintaining the Bcl-2 expression via upregulating the Brn-3a. Based on the above information, it is proposed that cotreatment with ginsenoside F1 and EGCG may rescue human keratinocytes through the similar mechanism to that of ginsenoside F1.

In our study, neither 5 μM ginsenoside F1 nor 50 μM EGCG alone inhibited the dephosphorylation of Rb induced by UVB irradiation, but importantly, the combination of 2 μM ginsenoside F1 and 10 μM EGCG inhibited the dephosphorylation of Rb in UVB-irradiated HaCaT cells. This suggests that other factors could certainly take part in the mechanisms involved in the prevention of apoptosis by adding the two agents together. Dephosphorylation of Rb has been reported to be associated with apoptosis[17]. The accumulation of hypophosphorylated Rb and its interaction with E2F are required for suppression of cell growth and induction of apoptosis[16]. It has been shown recently that Bcl-2 promoters hyperphosphorylaton of Rb in epithelial cells and blocks its apoptosis[20]. Despite meaningful of increase in Bcl-2 expression were observed in all cells treated with ginsenoside F1 and EGCG, either alone or in combination, the dephosphorylation of Rb was inhibited only by cotreatment with ginsenoside F1 and EGCG. Thus, it is conceivable that synergy between ginsenoside F1 and EGCG in protective effect from UVB-induced apoptosis may be mediated through a novel mechanism that modulate the phosphorylation state of Rb. Further studies are needed to precisely define the molecular mechanisms that underlie the synergistic action between ginsenoside F1 and EGCG. Nevertheless, our finding that cotreatment with ginsenoside F1 and EGCG reversed the adverse effect of UVB irradiation by preventing Rb dephosphorylation suggests that contreatment with these two agents is more effective treatment for prevention of HaCaT cells against UVB-induced apoptosis than treatment with either single agent.

In conclusion, these data demonstrate that low concentration cotreatment with ginsenoside F1 and EGCG successfully protects HaCaT cells from UVB-induced apoptosis by maintaining constant levels of Bcl-2, Brn-3a expressions and Rb phosphorylation state.

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