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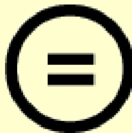

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A thesis

For the Degree of Master of Science in Medicine

Photo-protective effect of baicalein against ultraviolet B-induced oxidative stress

Min Chang Oh

Department of Medicine
Graduate School
Jeju National University

August, 2016

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자외선 B로 유도된 산화적 스트레스에 대한 baicalein의 광 보호효과

지도교수 현 진 원

오 민 창

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Photo-protective effect of baicalein against ultraviolet B-induced oxidative stress

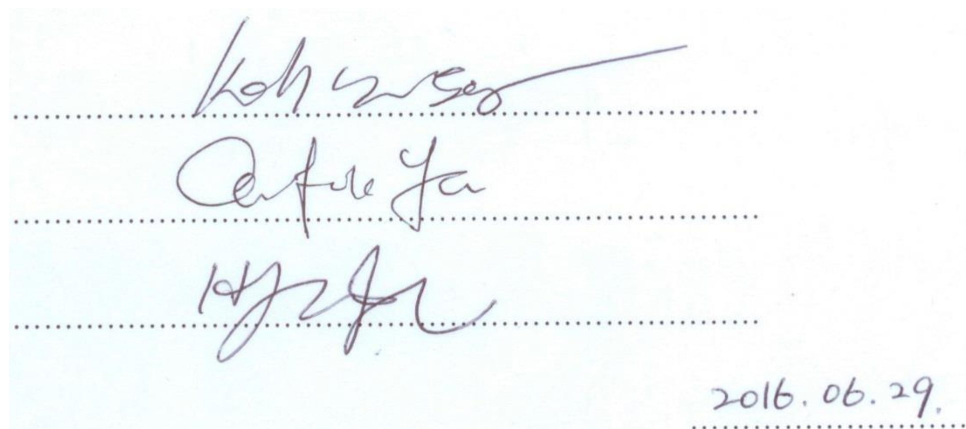
Min Chang Oh

(Supervised by Professor Jin-Won Hyun)

A thesis submitted in partial fulfillment of the requirement for the dgree of Master of Sceince in Medicine

2016. 08.

This thesis has been examined and approved.



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ABSTRACT

Baicalein (5,6,7-trihydroxy-2-phenyl-chromen-4-one) is a flavone, a type of flavonoid, originally isolated from the roots of *Scutellaria baicalensis*. This study evaluated the protective effects of baicalein against oxidative stress-mediated apoptosis induced by ultraviolet B (UVB) radiation in a human keratinocyte cell line (HaCaT). Baicalein absorbed light within the wavelength range of UVB. In addition, baicalein decreased the level of intracellular reactive oxygen species (ROS) in response to UVB radiation. Baicalein protected cells against UVB radiation-induced DNA breaks, 8-isoprostane generation and protein modification in HaCaT cells. Furthermore, baicalein suppressed the apoptotic cell death by UVB radiation. These findings suggest that baicalein protected HaCaT cells against UVB-induced cell damage and apoptosis by absorbing UVB radiation and scavenging ROS.

Keywords: Baicalein, Ultraviolet B, Oxidative stress, Reactive oxygen species, HaCaT cells

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All the main contents and experimental data of the thesis have been published in the journal as “*DOI: 10.4062/biomolther.2016.022, Biomolecules & therapeutics, June 2016*”, entitled “*Baicalein Protects Human Skin Cells against Ultraviolet B-Induced Oxidative Stress*”.

1. Introduction

Reactive oxygen species (ROS) are naturally produced in the body as a result of environmental or exposure normal metabolism. At high concentrations, ROS may induce oxidative stress to DNA, lipids, and proteins. Oxidation of these cellular substrates can cause degenerative diseases (Lee *et al*., 2014; Dhumrongvaraporn and Chanvorachote, 2013). Ultraviolet B (UVB) radiation can generate ROS including singlet oxygen, superoxide anion, and hydroxyl radical. These ROS can damage and oxidize cellular lipids, proteins, and DNA, leading to changes and photo-aging in skin (Palmer *et al*., 2010; Sklar *et al*., 2013). UVB radiation also have deleterious effects on the skin, including solar erythema, inflammation, premature aging, and carcinogenesis (Sime and Reeve, 2004; Narayanan *et al*., 2010; Lee and Park, 2014).

Recently, natural compounds have attracted attention as antioxidant because many synthetic compounds have toxic side effects; they show antioxidant effects via free radical scavenging capacity and/or enhancing antioxidant system (Sen *et al*., 2010; Vera Saltos *et al.*,

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2015). Baicalein (5,6,7-trihydroxyflavone), a flavone compound, is originally isolated from the roots of *Scutellaria baicalensis* and the aglycone of baicalin. It is also reported in *Oroxylum indicum*, also known as Indian trumpet flower. This flavonoid inhibits certain types of lipoxygenases and acts as an anti-inflammatory agent (Deschamps *et al*., 2006; Hsieh *et al*., 2007). It also has anti-proliferative effects on endothelin-1-induced proliferation of pulmonary artery smooth muscle cells via inhibition of transient receptor potential channel 1 expression (Lin *et al*., 2011). Our recent work showed that baicalein ameliorates mitochondrial oxidative stress via induction of manganese superoxide dismutase (Lee *et al*., 2011). Baicalein also protects cellular components against oxidative damage by scavenging ROS, inhibiting apoptosis and attenuates oxidative damage-induced expression of matrix metalloproteinase-1 (MMP-1) by regulating the mitogen-activated protein kinase pathway in HaCaT cells (Kim *et al*., 2012A). In addition, baicalein reduces oxidative damage-induced DNA damage by upregulating the DNA repair system (Kim *et al*., 2012B). However, UVB-induced oxidative damage has not been researched. Therefore, this study investigated HaCaT cells to evaluate whether baicalein protects cells against UVB-induced oxidative stress.

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2. Materials and Methods

2-1. Reagents

Baicalein, 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), Hoechst 33342, N-acetyl cysteine (NAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MTT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Diphenyl-1-pyrenylphosphine (DPPP) was obtained from Molecular Probes (Eugene, OR, USA). All other reagents and chemicals were of analytical grade.

2-2. Cell culture and UVB radiation

HaCaT cells were supplied by the Amore Pacific Company (Yongin, Republic of Korea) and maintained in an incubator at 37℃ in a humidified atmosphere containing 5% CO_2 plus 95% air. The cells were incubated in RPMI 1640 medium including 10% fetal bovine serum heated for 30 min at 56℃, penicillin (100 units/ml) and streptomycin (100 μg/ml). A CL-1000M UV Crosslinker (UVP, Upland, CA) was used as the UVB source, which delivers a UVB energy spectrum of 280–320 nm, and UVB dose is 30 mJ/cm².

2-3. Ultraviolet/visible absorption analysis

To observe the UVB absorption spectra of baicalein, which was diluted in DMSO (1:500), the compound was scanned by UV at 220–520 nm using Biochrom Libra S22 ultraviolet/visible spectrophotometer (Biochrom Ltd., Cambridge, UK).

2-4. Cell viability

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Cells were seeded in a 24well-plate at a density of 5 × 10^4 cells/well. Sixteen hours after seeding, cells were treated with baicalein at a concentration of 5, 10, 20, 30 μM or treating the cells with 1 mM NAC, 20 μM baicalein for 16 h, was followed 1 h later by 30 mJ/cm^2 of UVB. After incubating for 24 h at 37℃, 10 μl of MTT solution (2 mg/ml) was added to each well to yield a total volume of 500 μl and incubating cells for 4 h. Next, the plate was centrifuged at 1500 rpm for 5 min and the supernatants were removed. The formazan crystals were dissolved by 500 μl dimethyl sulfoxide (DMSO), and the absorbance at 540 nm was measured by using aspectrophotometer.

2-5. DPPH radical scavenging activity

Baicalein (10, 20, 30 μM) and 1mM NAC was added to a solution of 0.1 mM DPPH in methanol. The resulting reaction mixture was shaken vigorously. 3 h later, the amount of residual, unreacted DPPH was detected at 520 nm by a scanning multi-well spectrophotometer.

2-6. Detection of hydroxyl radical

Hydroxyl radical generated by the Fenton reaction ($FeSO_4$+H_2O_2) was reacted with DMPO. The resultant DMPO/·OH adducts was detected using an ESR spectrometer (Li *et al*., 2004). The ESR spectrum was measured 2.5 min after a phosphate buffer solution (pH 7.4) was reacted with 20 μl each of 10 mM H_2O_2, 0.3 M DMPO and 20 μM baicalein. The ESR spectrometer parameters were set as follows: power=1.00 mW; central magnetic field=336.8 mT; modulation width=0.2 mT; sweep width=10 mT; frequency=9.4380 GHz; sweep time=0.5 min; amplitude=600; gain=200, time constant=0.03 sec; temperature=25°C.

2-7. Detection of superoxide anion

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Superoxide anion generated by the xanthine/xanthine oxidase system was reacted with DMPO, and the resultant DMPO/·OOH adducts was detected using an ESR spectrometer. ESR signaling was measured 2.5 min after 20 μl of xanthine oxidase (0.25 U/ml) was mixed with 20 μl each of 10 mM xanthine, 3 M DMPO and 20 μM baicalein. The ESR spectrometer parameters were set as follows: power=1.00 mW; central magnetic field=336.8 mT; modulation width=0.2 mT; sweep width=10 mT; frequency=9.4380 GHz; sweep time=0.5 min; amplitude=600; gain=500, time constant=0.03 sec; temperature=25°C.

2-8. Measurement of intracellular ROS

The DCF-DA method was determined to measure the levels of intracellular ROS (Rosenkranz *et al*., 1992). Cells were seeded in a 96-well plate at 1×10^4 cells per well and at 16 h after plating, cells were treated with baicalein at the concentration of 5, 10, 20 and 30 μM/ml for 1 h. 1 mM H_2O_2 or UVB radiation (30 mJ/cm²) was then treated to the medium, and cells were incubated for an additional 24 h at 37℃. After the treatment of 25 μM DCF-DA for 10 min, the fluorescence of DCF-DA was detected using a Perkin-Elmer LS-5B spectrofluorometer. The scavenging effect of ROS generation (in percent) was calculated as [(fluorescence value of H_2O_2 or UVB-treated cells alone) − (fluorescence value of H_2O_2 or UVB-treated cells with baicalein treatment) / (fluorescence value of H_2O_2 or UVB-treated cells alone)] × 100. For imaging analysis of the generation of intracellular ROS, cells were seeded on a four-well chamber slide at a density of 2×10^5 cells/ml. At 16 h after plating, cells were treated with baicalein at the concentration of 20 μM for 1 h. UVB (30 mJ/cm²) was exposed to the plate and cells were incubated for an additional 24 h at 37℃. And then, 100 μM of DCF-DA was treated to each well, and the cells were incubated at 37℃. After 30 min, cells were washed by PBS, the stained cells were mounted on the chamber slide with mounting medium (DAKO). The images were obtained on a confocal microscope using the

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Laser Scanning Microscope 5 PASCAL software (Carl Zeiss).

2-9. Single-cell gel electrophoresis (Comet assay)

The degree of oxidative DNA damage was used in a Comet assay (Rajagopalan *et al.*, 2003). The cell mixture was mixed with 75 μl of 0.5% low-melting agarose (LMA) at 39℃ and the mixture was spread on a fully frosted microscopic slide pre-coated with 200 μl of 1% normal melting agarose (NMA). After the agarose was solidified, the slide was covered with another 75 μl of 0.5% LMA and then immersed in a lysis solution (10% DMSO, 10 mM Tris, 2.5 M NaCl, 1% Trion X-100, 100 mM Na-EDTA and pH 10) for 1 h at 4℃. The slides were subsequently placed in a gel electrophoresis apparatus containing 10 mM Na-EDTA (pH 13) and 300 mM NaOH for 40 min to allow for the expression of alkali-labile damage and DNA unwinding. An electrical field was then applied (300 mA, 25 V) for 20 min at 4℃ to draw the negatively charged DNA towards the anode. The slides were washed three times for 5 min at 4℃ in a neutralizing buffer (0.4 M Tris, pH 7.5), stained with 75 μl of propidium iodide (20 μg/ml) and observed under a fluorescence microscope and an image analyzer (Kinetic Imaging, Komet 5.5, UK). The percentage of the total fluorescence in the comet tails and the tail lengths of 50 cells per slide were recorded.

2-10. Lipid peroxidation assay

Lipid peroxidation was assayed by colorimetric detection of 8-isoprostane, a stable end-product of lipid peroxidation, in the conditioned medium of HaCaT cells (Beauchamp *et al.*, 2002). A commercial enzyme immune assay (Cayman Chemical, Ann Arbor, MI, USA) was used according to the manufacturer's instructions to detect 8-isoprostane levels. In addition, lipid peroxidation was also estimated by use of diphenyl-1-pyrenylphosphine (DPPP), a fluorescent probe (Okimoto *et al*., 2000). Cells were treated with 20 μM baicalein. After 1 h,

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UVB (30 mJ/cm²) was exposed. 5 h later, 5 mM DPPP was added and incubated for 30 min in the dark. The DPPP fluorescence images were measured by a Zeiss Axiovert 200 inverted microscope at an excitation wavelength of 351 nm and an emission wavelength of 380 nm.

2-11. Protein carbonyl formation

Cells were treated with 20 μM baicalein for 1 h, followed by the radiation of UVB (30 mJ/cm²) and further incubation for 36 h. The amount of protein carbonyl formation was analyzed by using an Oxiselect™ protein carbonyl enzyme-linked immunosorbent assay (ELISA) kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's instructions.

2-12. Nuclear staining with Hoechst 33342

Cells were treated with baicalein at a concentration of 20 μM or 1 mM NAC and exposed to UVB radiation (30 mJ/cm²) into plate 1 h later. After 24 h incubation, the DNA specific fluorescent dye Hoechst 33342 (1.5 μl of a 10 mg/ml stock) was treated to each well and the cells were incubated. After 10 min, the stained cells were observed under a fluorescence microscope equipped with a Cool SNAP-Pro color digital camera. The degree of nuclear condensation was measured and the apoptotic cells were quantified. The apoptotic index was calculated as follows: (number of apoptotic cells in baicalein-treated group/total number of cells in baicalein-treated group)/(number of apoptotic cells in control group/total number of cells in control group).

2-13. Statistical analysis

All measurements were performed in triplicate and all values are expressed as the mean ± standard error. The results were subjected to an analysis of variance (ANOVA) using

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Tukey's test to analyze differences between means. In each case, a p value < 0.05 was considered statistically significant.

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3. Results

3-1. UVB absorption by baicalein

Absorption of UVB by baicalein was determined using an ultraviolet (UV)/visible spectrophotometer. Baicalein showed peak absorption at 270 nm, which is close to the range of UVB (280–320 nm) (Fig. 1). Therefore, light absorption by baicalein might be closely associated with its cytoprotective effect against UVB radiation.

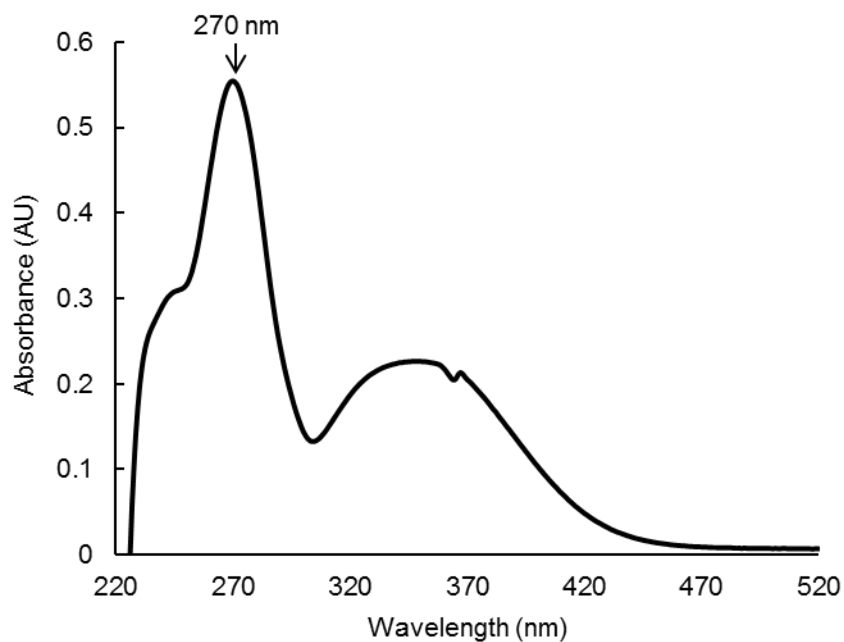

Figure 1. Effect of baicalein on UVB absorption. UV/visible spectroscopy was conducted in

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the spectral range from 220 to 520 nm. The peak absorption is at 270 nm.

3-2. Baicalein reduces ROS generation

The MTT assay revealed that baicalein itself did not have cytotoxic effects on HaCaT cells at any concentrations used up to 30 μM (Fig. 2A). Baicalein scavenged the DPPH radical in a concentration-dependent manner; 1% of radicals were scavenged at 10 μM, 3% at 20 μM, and 11% at 30 μM. By comparison, the well-known ROS scavenger N-acetyl cysteine (NAC) scavenged 91% of radicals at a concentration of 1 mM (Fig. 2B). Moreover, we used electron spin resonance spectrometry to measure the ability of baicalein to scavenge hydroxyl radical and superoxide anion. In the $FeSO_4 + H_2O_2$ system ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$), DMPO/·OH adducts yielded signals of 3,802 in the absence of baicalein and 1,376 in the presence of 20 μM baicalein (Fig. 2C). Similarly, in the xanthine/xanthine oxidase system, DMPO/·OOH yielded signals of 3,671 in the absence of baicalein and 2,045 in the presence of 20 μM baicalein (Fig. 2D), indicating that baicalein can scavenge superoxide anion. Next, the intracellular ROS-scavenging activity of baicalein after H_2O_2 treatment or UVB radiation was determined using the DCF-DA assay (Fig. 2E). Fluorescence spectrometry data revealed that the intracellular ROS-scavenging activity of baicalein in H_2O_2-treated cells was 40% at 5 μM, 48% at 10 μM, 56% at 20 μM, and 60% at 30 μM, compared with 52% for 1 mM NAC, and in UVB-exposed cells was 12% at 5 μM, 14% at 10 μM, 19% at 20 μM, and 21% at 30 μM, compared with 18% for 1 mM NAC (Fig. 2E). Furthermore, confocal microscopy revealed that 20 μM baicalein ameliorated the increase in intensity (green color) caused by UVB radiation (Fig. 2F), indicating that baicalein treatment reduces ROS generation and that this compound possesses ROS-scavenging properties. Based on the results of these experiments, we chose to use the optimal dose of 20 μM baicalein for subsequent investigations.

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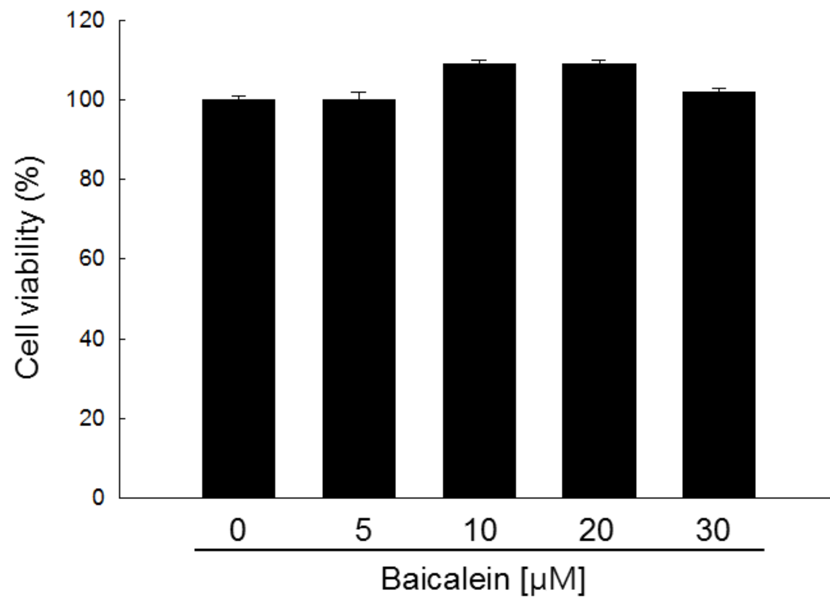

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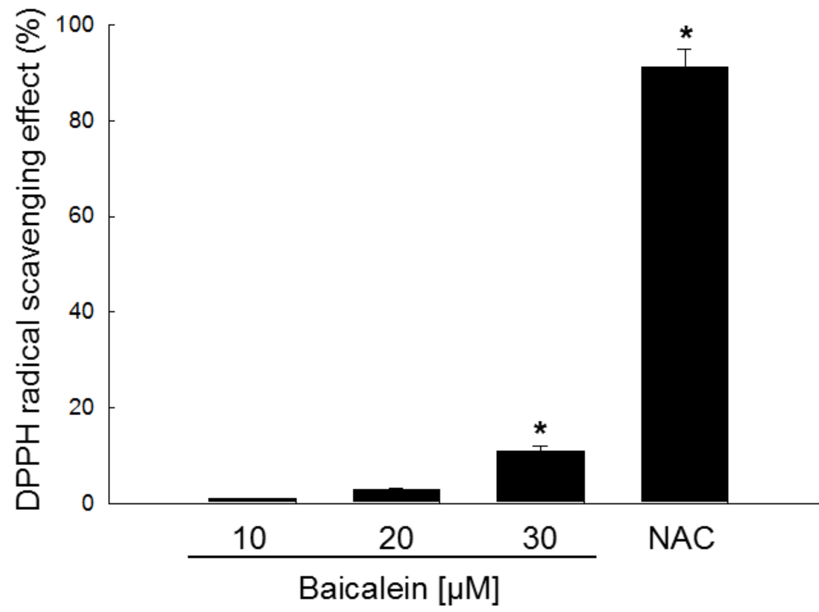

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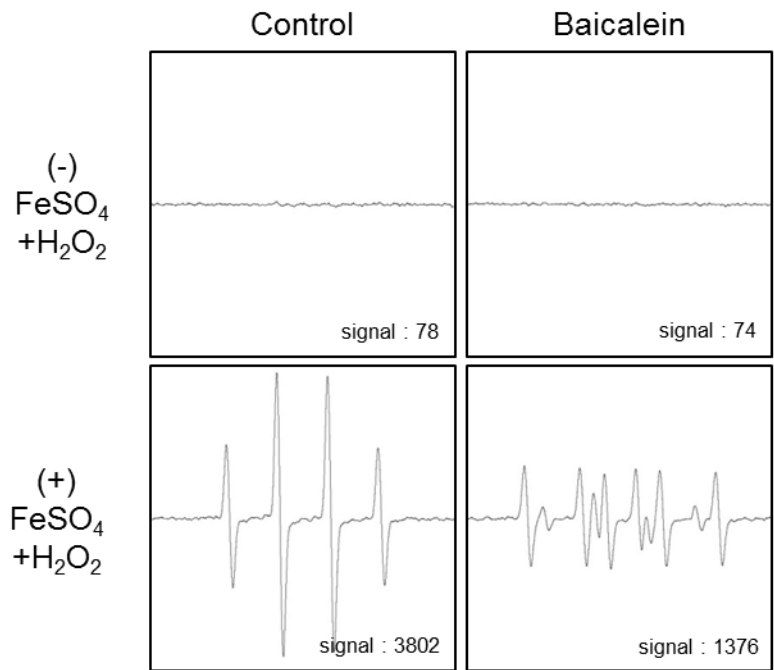

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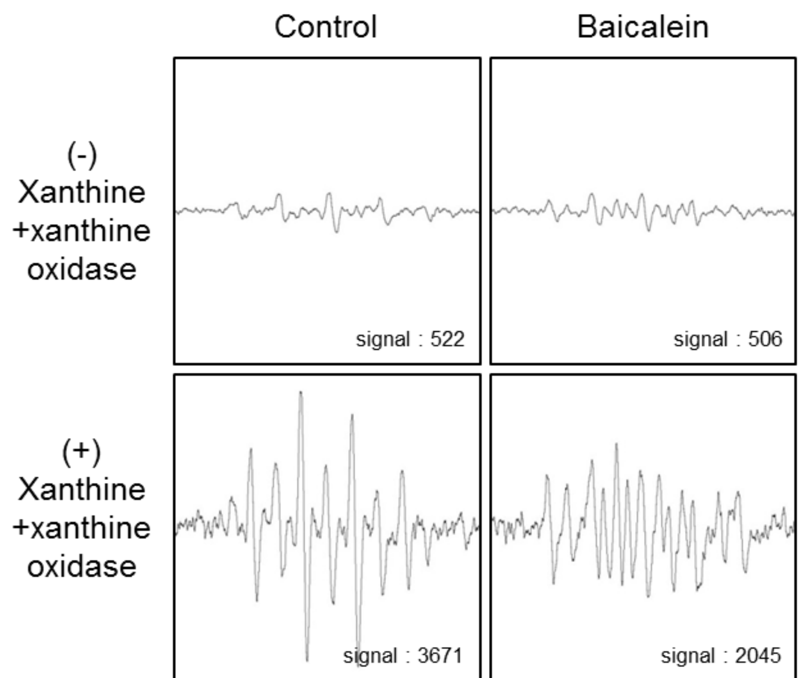

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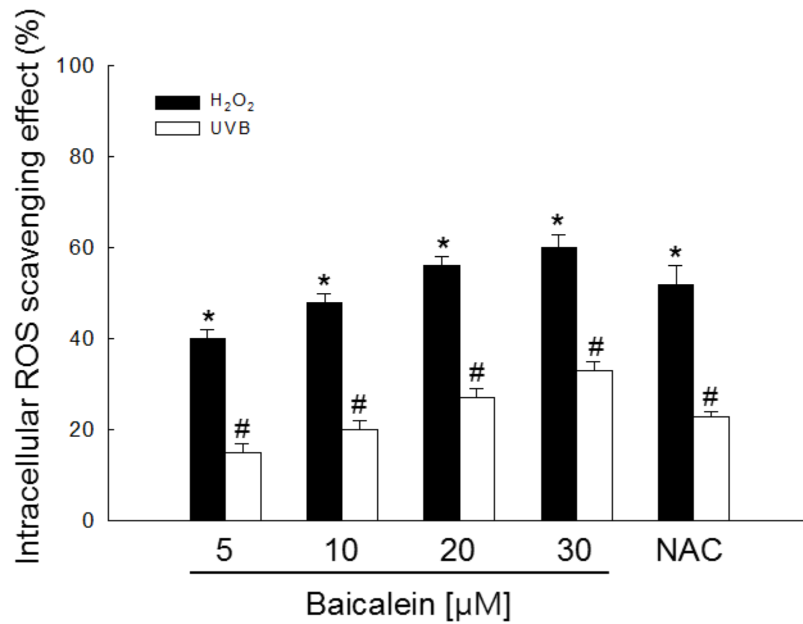

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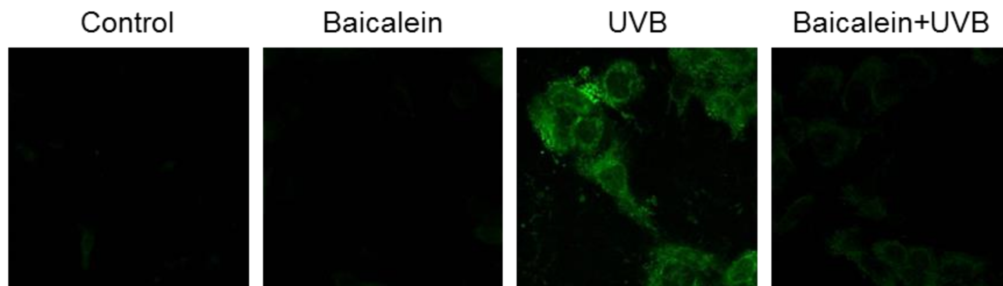

Figure 2. Baicalein reduces ROS generation. (A) HaCaT cell viability was investigated using the MTT assay to determine the cytotoxic effects of baicalein. (B) The radical-scavenging effects of baicalein were investigated using the DPPH assay. (C) The ability to scavenge hydroxyl radical at 20 μM baicalein was estimated using the Fenton reaction ($FeSO_4$ + H_2O_2 system). (D) The ability to scavenge superoxide anion at 20 μM baicalein

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was evaluated using the xanthine/xanthine oxidase system. (E) Cells were treated with 5, 10, 20, or 30 μM baicalein or 1 mM NAC. One hour later, cells were irradiated with UVB or treated with 1 mM H_2O_2. After 30 min, cells were stained with DCF-DA and intracellular ROS were measured using a spectrophotometer. $^{*,\#}$Significantly different from control cells of H_2O_2 or UVB radiation, respectively ($p < 0.05$). (F) Representative confocal images illustrate that UVB radiation increased the fluorescence intensity of DCF (produced by ROS) compared with the control, and baicalein treatment of UVB-exposed cells reduced the fluorescence intensity (green color).

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3-3. Baicalein protects DNA, lipids, and proteins against UVB-induced oxidative damage

We next investigated whether baicalein can inhibit damage to macromolecules in UVB-exposed cells. First, we monitored UVB-induced DNA damage using the comet assay. The length of comet tails in microscopy images and the percentage of cellular fluorescence in tails are shown in Figure 3A. After treatment of cells with UVB radiation, the comet tail length was distinctly elongated, as well as the ratio of damaged DNA outside of nuclei. Treatment of UVB-exposed cells with baicalein clearly reduces the percentage of damaged DNA in comet tails from 52% to 30%. Second, we examined the level of 8-isoprostane, a hallmark of lipid peroxidation, which is released by cells into the culture medium upon oxidative damage. Cells exposed to UVB secreted a higher level of 8-isoprostane than untreated cells; however, pretreatment of UVB-exposed cells with baicalein significantly reduced the 8-isoprostane level (Fig. 3B). In addition, lipid peroxidation was investigated by examining the fluorescent product DPPP oxide produced from DPPP. The intensity of DPPP oxide was higher in UVB-exposed cells than in control cells (blue color). Pretreatment of UVB-exposed cells with baicalein reduced the fluorescence intensity (Fig. 3C). Finally, we measured protein carbonylation, which is a reaction that occurs during the process of protein oxidation to form carbonyl groups (Pirinccioglu *et al.*, 2010). UVB irradiation obviously increased the level of carbonyl moieties, whereas pretreatment of UVB-exposed cells with baicalein notably suppressed the formation of protein carbonyls (Fig. 3D). Taken together, these results indicate that baicalein effectively protects DNA, lipids, and proteins against oxidative damage induced by UVB radiation.

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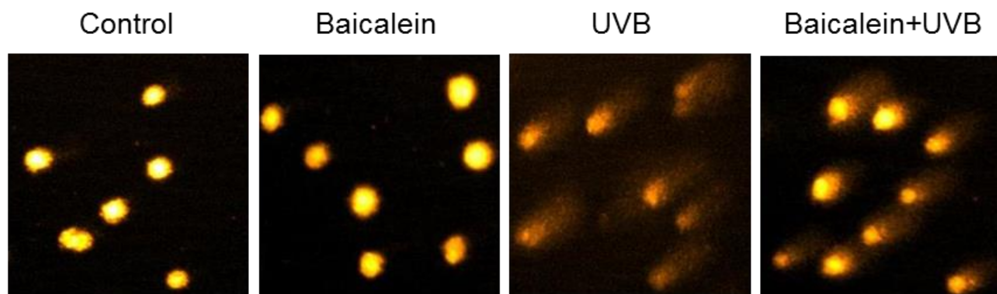

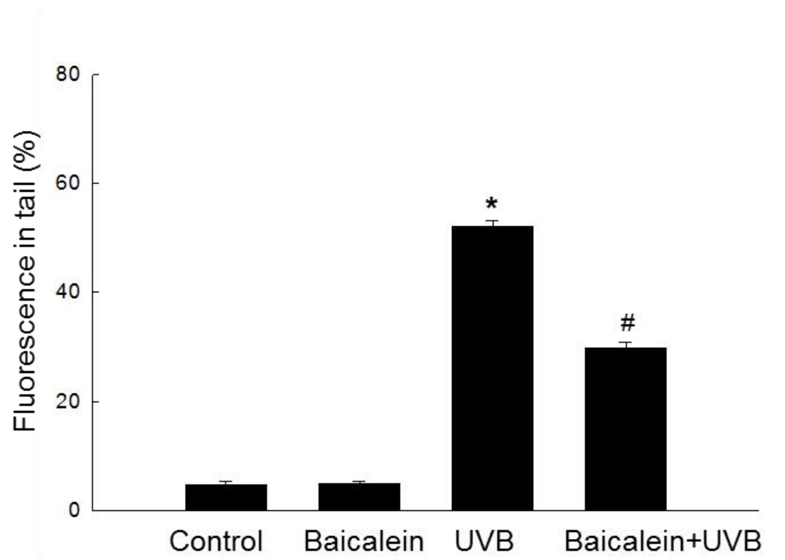

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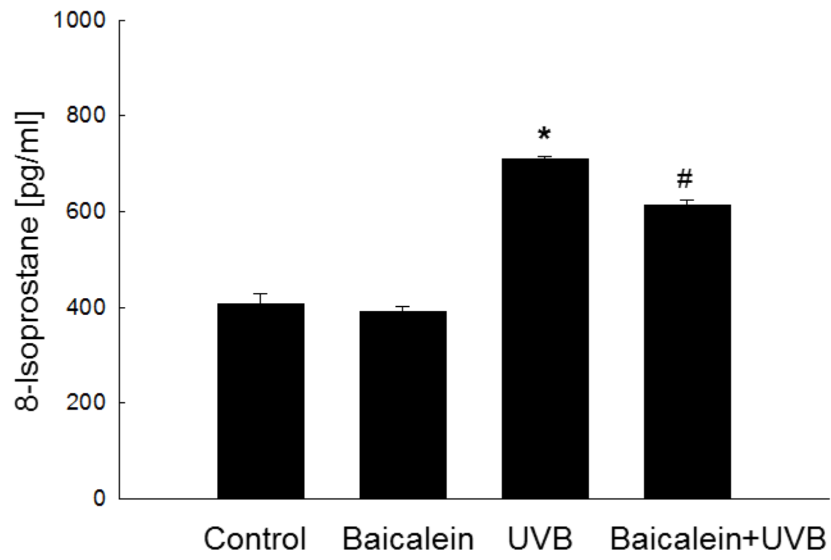

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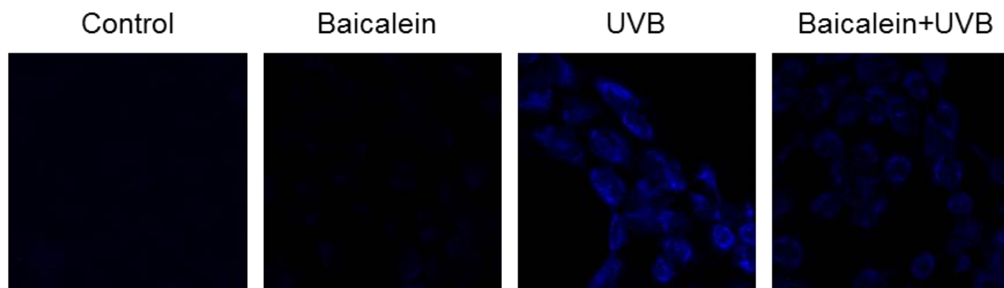

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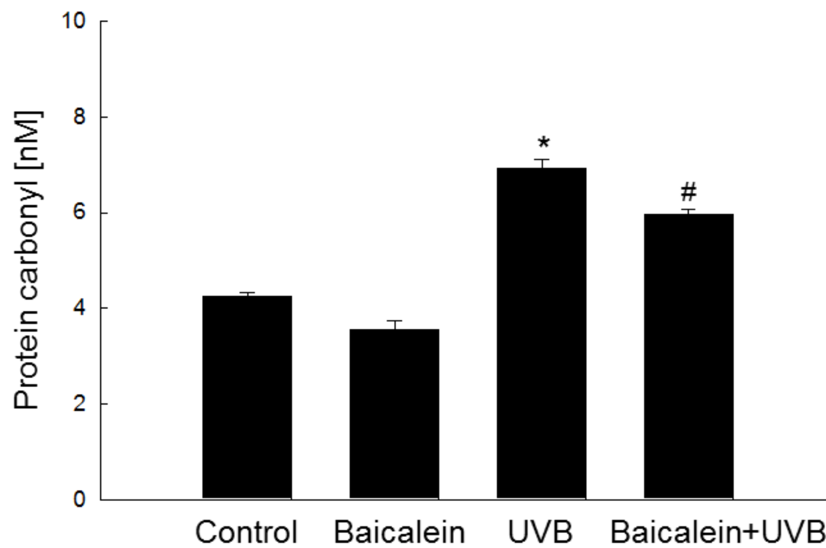

Figure 3. Baicalein protects cells against UVB-induced DNA damage, lipid peroxidation, and protein carbonylation. (A) The comet assay was performed to assess DNA damage. Representative images and percentage of cellular fluorescence within comet tails are shown. *Significantly different from control cells ($p < 0.05$) and #significantly different from cells only exposed to UVB radiation ($p < 0.05$). (B) Cells were treated with 20 μM baicalein. After 1 h, cells were exposed to UVB radiation. After incubation for a further 24 h, lipid peroxidation was determined using an 8-isoprostane enzyme immunoassay kit. *Significantly different from control cells ($p < 0.05$) and #significantly different from cells only exposed to UVB radiation ($p < 0.05$). (C) Lipid peroxidation was detected using a confocal microscope after DPPP staining. (D) Protein oxidation was assayed by measuring the amount of carbonyl formation. *Significantly different from control cells ($p < 0.05$) and #significantly different from cells only exposed to UVB radiation ($p < 0.05$).

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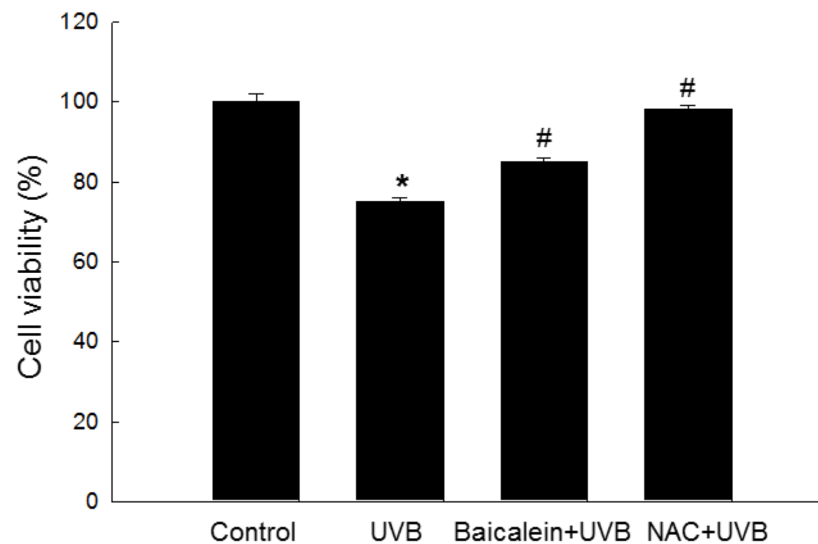
3-4. Baicalein suppresses apoptosis induced by UVB radiation

To elucidate the cytoprotective effect of baicalein against UVB-induced apoptosis, we examined the viability of HaCaT cells exposed to UVB, pretreated or not with 20 μM baicalein. The viability of UVB-exposed cells was reduced to 75% relative to control cells; however, pretreatment with baicalein increased viability to 85% compared to 95% of NAC (Fig. 4A). Nuclei were stained with Hoechst 33342, and then cells were assessed by microscopy. Normal nuclei were observed in control and baicalein-treated cells, whereas significant nuclear condensation was observed in UVB-exposed cells (apoptotic index = 30). However, when these cells were pretreated with baicalein or NAC, nuclear fragmentation was reduced (baicalein, apoptotic index = 9.8; NAC, apoptotic index = 9.2) (Fig. 4B). These results indicate that baicalein protects cells against UVB-induced apoptosis.

A



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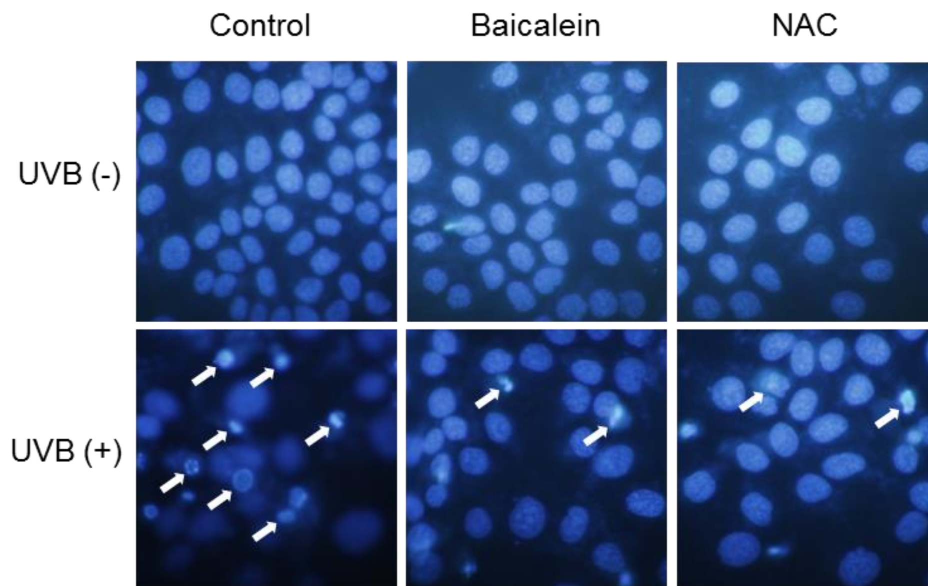

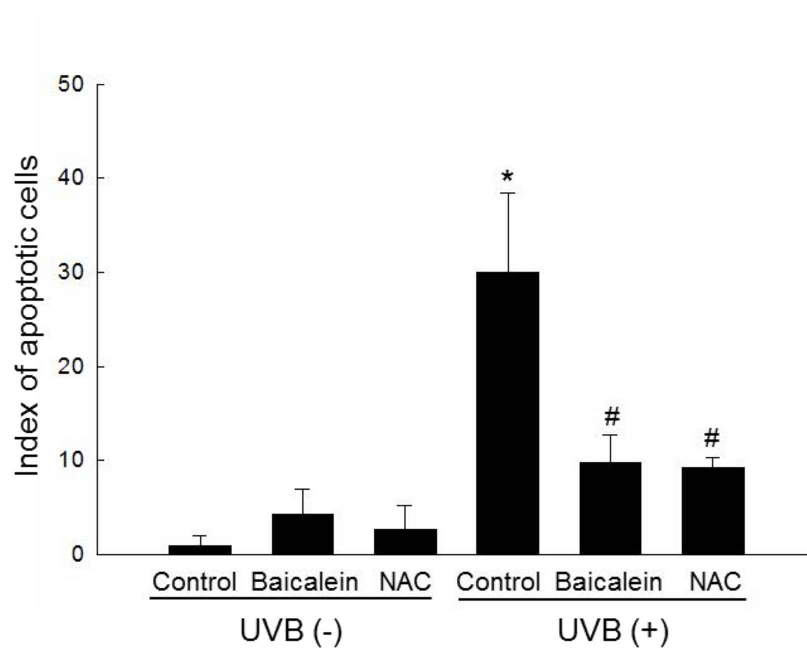

Figure 4. Baicalein protects cells against apoptosis induced by UVB. (A) Cells were treated with 20 μM baicalein and exposed to UVB radiation 1 h later. After 24 h incubation, cell

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viability was determined by the MTT assay and is expressed as a percentage of the control. 1 mM NAC was used as a positive control. *Significantly different from control cells ($p < 0.05$) and #significantly different from cells only exposed to UVB radiation ($p < 0.05$). (B) Cells were stained with Hoechst 33342 and detected by fluorescence microscopy. Apoptotic bodies were quantitated. 1 mM NAC was used as a positive control. *Significantly different from control cells ($p < 0.05$) and #significantly different from cells only exposed to UVB radiation ($p < 0.05$).

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4. Disccusion

In the present study, we focused on the protective effects of baicalein against UVB radiation-induced oxidative stress. Our results indicated that baicalein absorbed UV photons, that was mostly caused by due to its polyphenolic constituents. Spectral data of natural phenols show a typical UV absorbance characteristic of benzene aromaticity at 270 nm. Baicalein is also natural phenol; therefore, its UV light absorbance (peak 270 nm) may be via benzene aromaticity (Jean-Denis *et al*., 2006).

UVB radiation induces ROS generation such as singlet oxygen, superoxide anion, hydroxyl radical and peroxy radical. In the present study, baicalein scavenged ROS such as superoxide anion and hydroxyl radical in cell free system, and intracellular ROS induced by treatment with H_2O_2 or UVB radiation. The distinct pathways by which flavonoid molecules (ArOH) transfer their charge provide several mechanisms of their antioxidant action. The representative one leads to the direct O-H bond breaking and proceeds by rapid donation of the proton and electron to a radical form (ArOH + HO· → ArO· + HOH), while the second one assumes indirect H atom abstraction (ArOH + HO· → $ArOH^+$ + OH· → ArO· + HOH) (Jasmina *et al*., 2011). Baicalein has O-H bonds, and it can scavenge radical by O-H bonds.

The UVB-induced ROS induce chemical modifications in DNA by the formation of purine/pyrimidine dimers and strand breaks leading to mutagenesis and loss of normal cellular metabolic functions. And they can damage lipids, producing lipid peroxides that are converted to products such as 8-isoprostane, malondialdehyde and conjugated dienes (Petrova *et al.*, 2011; Schneider *et al.*, 2006). Furthermore, UVB-induced oxidative stress damages proteins by site-specific modifications of amino acids, aggregation of cross-linked reaction products, increased susceptibility to proteolysis, fragmentation of peptide chains,

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and oxidation of specific amino acids (Rajesh and Datta, 2015). Therefore, UVB-induced oxidative stress triggers cell damage. However, baicalein protects cellular components such as DNA, lipids, and proteins against UVB-induced oxidative damage. The oxidative stress by UVB radiation mediates apoptosis via the release of mitochondrial cytochrome c into the cytosol and activation of apoptosis-related caspases (Pallela *et al*., 2010). However, baicalein protects cells against UVB-induced apoptotic cell death.

In conclusion, baicalein protects against UVB-induced oxidative stress via scavenging ROS, inhibiting apoptosis, and absorbing UV photons. In a future study, we will elucidate the mechanisms by which baicalein prevents ROS generation and inhibits apoptosis.

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6. Abstract in Korean

Baicalein (5,6,7-trihydroxy-2-phenyl-chromen-4-one) 은 하나의 플라본이자 플라보노이드의 한 타입으로, 황금 (*Scutellaria baicalensis*) 의 뿌리로부터 추출되었다. 이 연구는 인간 각질세포 (HaCaT) 에서 자외선 B 의 조사에 의해 유도된 산화적 스트레스 매개 세포사멸에 대한 baicalein 의 보호효과를 평가하였다. Baicalein 은 자외선 B 의 파장 범위 내의 빛을 흡수했다. 또한, baicalein 은 자외선 B 의 조사에 반응하여 세포 내 활성산소종 (ROS) 의 수준을 감소시켰다. Baicalein 은 HaCaT 세포에서 자외선 B 의 조사에 유도된 DNA 손상, 8-isoprostane 발생과 단백질 변형에 대하여 세포를 보호했다. 게다가, baicalein 은 자외선 B 조사에 의한 세포사멸을 감쇠시켰다. 이러한 연구결과는 baicalein 이 자외선 B 로 유도된 세포손상과 세포사멸을 자외선 B 조사를 흡수하고 ROS 를 소거함으로써 HaCaT 세포를 보호한다는 것을 시사한다.

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7. Acknowledgement

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후배 유진, 이제 시작될 석사과정 힘들겠지만 멋지게 졸업하고 내 뒤를 잇길 기대하마.

그 외에도 도와주신 분들이 많이 남았지만 한 분 한 분 감사의 말씀을 전하지 못하여 죄송합니다. 감사의 말씀을 전하지 못한 분들께도 진심으로 감사하다는 말씀 드리고 싶습니다. 계속해서 성장하는 오민창 되겠습니다. 감사합니다!

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