European Journal of Pharmacology 661 (2011) 124–132

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and Inflammation

Effects of baicalein and wogonin isolated from *Scutellaria baicalensis* roots on skin damage in acute UVB-irradiated hairless mice

Yoshiyuki Kimura [a,*], Maho Sumiyoshi [b]

[a] Division of Biochemical Pharmacology, Department of Basic Medical Research, Ehime University Graduate School of Medicine, Shitsukawa, Toon City, Ehime 791-0295, Japan
[b] Division of Functional Histology, Department of Functional Biomedicine, Ehime University Graduate School of Medicine, Shitsukawa, Toon City, Ehime 791-0295, Japan

ARTICLE INFO

Article history:
Received 4 September 2010
Received in revised form 28 March 2011
Accepted 14 April 2011
Available online 28 April 2011

Keywords:
Ultraviolet B
MMP-9
VEGF
COX-2
Wogonin
Baicalein

ABSTRACT

Solar ultraviolet (UV) radiation causes skin damage including increases in skin thickness, edema, and flush. In this study, we examined the effects of two main flavonoids (wogonin and baicalein) isolated from the roots of *Scutellaria baicalensis*, a traditional remedy for allergic inflammatory diseases long used in China and Japan, on acute UVB irradiation-induced skin damage in hairless mice. Baicalein and wogonin (10 or 50 mg/kg) were administered orally twice daily for 14 consecutive days. The UVB irradiation was performed at a dose of 200 mJ/cm^2 on days 7 and 8 of the treatment with the two main flavonoids. Baicalein, and wogonin prevented the increases in skin thickness and the levels of matrix metalloproteinase (MMP)-9, and vascular endothelial growth factor (VEGF) induced by the irradiation. Wogonin reduced the levels of cyclooxygenase (COX)-2 and hypoxia inducible factor (HIF)-1α in UVB-treated HaCaT cells. These findings suggest that wogonin inhibits irradiation-induced skin damage by suppressing increases in the levels of MMP-9, and VEGF through the inhibition of COX-2 and HIF-1α expression. Baicalein inhibited COX-2 and NF-κB/p65 expression, but stimulated HIF-1α expression. Therefore, its inhibitory action is likely due to the expression of MMP-9 and VEGF through the suppression of COX-2 and NF-κB/p65 expression. Furthermore, the inhibitory effects of baicalein on UVB-irradiated hyperplasia of skin epidermis may be due to the stimulation of HIF-1α expression.

1. Introduction

Since ancient times, the roots of *Scutellaria baicalensis* Georgi (Labiatae) have been used to treat allergic and inflammatory diseases in China and Japan. It is well established that the symptoms of skin aging, such as wrinkles and pigmentation, develop earlier in sun-exposed skin than in unexposed skin: a phenomenon referred to as photoaging. Ultraviolet (UV) B radiation is an important environmental factor because of its hazardous effect on health, which includes the generation of skin cancers (de Gruijl et al., 1993), suppression of the immune system (Beissert and Schwarz 1999), and premature skin aging (Fisher et al., 1997). In a series of studies on the effects of natural products on UV-induced skin damage, we found that the oral administration of turmeric extract (Sumiyoshi and Kimura 2009), and an olive leaf extract and its component oleuropein (Kimura and Sumiyoshi 2009; Sumiyoshi and Kimura 2010) prevented increases in skin thickness induced by acute or chronic UVB irradiation in mice. Inomata et al. (2003) reported that metalloproteinase (MMP)-2 and MMP-9 were stronger in wrinkle-bearing skin, and they showed increases as detected by gelatin zymography in chronic UVB-exposed skin. Yano et al. (2002, 2004) reported that skin vascularization was increased after acute and chronic UVB exposure with a significant increase in both the number and the size of dermal blood vessels, associated with up-regulation of vascular endothelial growth factor (VEGF) expression in the hyperplastic epidermis. Cyclooxygenase-2 (COX-2) plays important roles in the development of carcinogenesis as well as inflammation in UVB-irradiated skin (Cui et al., 2004; Grandjean-Laquerrierre et al., 2002; Kimura and Sumiyoshi, 2009; Nijsten et al., 2004; Surth, 2003; Wilgus et al., 2003). Thus, UVB-induced skin carcinogenesis and inflammation might be closely associated with the systems of MMP, VEGF, and COX-2 expression (Aggarwal et al., 2006). The hypoxia inducible factor (HIF)-1α binds to specific promoter moieties of genes encoding erythropoietin, VEGF, glycolytic transporters (Ben-Av et al., 1995; Paleolog et al., 1998; Wenger and Gassmann, 1997). The nuclear transcription factor nuclear factor kappa B (NF-κB) is known to play a critical role in the pathogenesis of cancer, inflammation and many other pathological conditions (Sen and Packer 1996; Siebenlist et al. 1994; Waddick and Uckun 1999). It is well known that the NF-κB plays a key role in skin biology and development of cancer (Kaufman and Fuchs, 2000). Thus, skin damage caused by UVB irradiation, including skin thickness is closely associated with the increase in expression of MMP-2, MMP-9, VEGF, COX-2, HIF-1α, and NF-κB activation. In this study, we examined the effects of the oral administration of two main flavonoids (baicalein and wogonin) isolated form *S. baicalensis* roots on the various parameter including MMP-2, MMP-9 and VEGF expression of skin caused by UVB irradiation in *in vivo* model. To clarify the inhibitory mechanism of

* Corresponding author. Tel.: +81 89 960 5922; fax: +81 89 960 5239.
E-mail address: yokim@m.ehime-u.ac.jp (Y. Kimura).

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doi:10.1016/j.ejphar.2011.04.033

baicalein and wogonin on the UVB-irradiated skin damage, we further examined the effects of baicalein and wogonin on COX-2, nuclear HIF-1α and NF-κB/p65 expression induced by UVB irradiation in human keratinocyte cell line HaCaT cells (*in vitro*).

2. Materials and methods

2.1. Materials

The dried roots of *S. baicalensis* Georgi (Labiatae) were purchased from Mikuni Co. (Osaka Japan). The two main flavonoids such as baicalein and wognin were isolated from ethylacetate and methanol extracts of the dried roots according to a method described previously (Kimura et al., 1982, 1984). The structure of wogonin and baicalein is shown in Fig. 1. All chemicals used in this study were of reagent grade and purchased from Wako Pure Chemical Co. (Osaka, Japan). The culture medium, Dulbecco's modified Eagle's medium (DMEM) was obtained from Nissui Pharmacy (Tokyo, Japan). Antibiotic and antimycotic solutions (100×) containing 10,000 units of penicillin, 10 mg/ml of streptomycin, and 25 μg/ml of amphotericin B in 0.9% NaCl were purchased from Sigma Co. (St. Louis, MO). Fetal bovine serum (FBS) was purchased from Gibco BRL (Auckland, New Zealand). The 100-mm culture dishes were purchased from Corning Glass Works (NY). A mouse VEGF enzyme-linked immunosorbent assay (ELISA) kit and tissue protein extraction reagent were purchased from R&D Systems (Minneapolis, MN) and Pierce Co. (Rockford, IL), respectively. The rabbit polyclonal anti-human cyclooxygenase (COX)-2, rabbit polyclonal anti-NF-κB/p65, mouse monoclonal anti-HIF-1α, and anti-β-actin antibodies were purchased from Cell Signaling Technology Inc. (MA), Sigma Co., and BD Bioscience (CA), respectively.

2.2. Animals

Male albino hairless HOS: HR-1 mice (5 weeks old) were purchased from Hoshino laboratory Animals Co. Ltd. (Saitama, Japan), housed for 1 week in a temperature-controlled room at 25 ± 1 °C and 60% relative humidity, and given free access to a standard laboratory diet and water during this experiment. Mice were treated according to the Ethical Guidelines of the Animal Center, Graduate School of Medicine, Ehime University, and the experimental protocol was approved by the Animal Studies Committee of Ehime University.

2.3. Measurement of skin thickness and elasticity, and ear thickness in acute UVB-irradiated mice

A UVB lamp (15 W; maximum wavelength 312 nm; intensity 100 μW/cm^2; Ieda Boueki Co., Tokyo, Japan) was used to examine the effects of the two main flavonoids (wogonin and baicalein) isolated from *S. baicalensis* roots on skin thickness and elasticity following acute irradiation. The period of irradiation was varied to control the amount of UVB energy applied to the dorsal region of each animal. The minimal erythema dose (MED) was about 36 mJ/cm^2. Wogonin and baicalein (10 or 50 mg/kg) were administered orally twice daily for 14 consecutive days. The UVB irradiation was performed at a dose of 200 mJ/cm^2 on days 7 and 8 after the oral administration of the flavonoids started. Skin thickness was assessed by measuring skin-fold thickness as described (Kimura and Sumiyoshi, 2009; Park et al., 2006). Briefly, dorsal skin of the hairless mice was lifted up by pinching gently under anesthetization with pentobarbital and skin-fold thickness was measured using a Quick Mini caliper (Mitsutoyo Co., Kanagawa, Japan). Ear thickness was measured by pinching gently using a Quick Mini caliper. Skin and ear thickness after UVB irradiation were measured every other day. On day 15, the mice were killed by cervical dislocation and irradiated skin was removed for analysis.

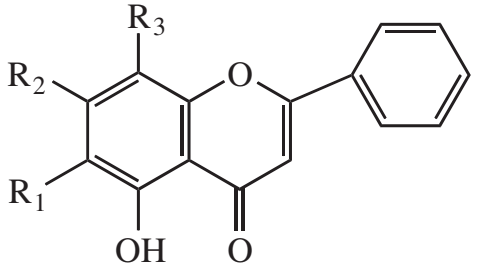

Fig. 1. The structure of baicalein and wogonin isolated from *S. baicalensis* roots.

2.4. Measurement of diameter and length of blood vessels in UVB-irradiated skin

The removed dorsal skin was washed in PBS (pH 7.0), and the subcutaneous blood vessels were photographed using a stereoscopic microscope. The diameter of the blood vessels was measured with Digimatic calipers (Mitsutoyo, Kanagawa, Japan). The length of all blood vessels was measured suing a Coordinate Area and Culvimeter machine (X-Plan 360 dII; Ushitaka, Tokyo, Japan), and the length of blood vessels was expressed as length mm/cm^2 field.

2.5. Measurement of vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-2, and MMP-9 expression in UVB-irradiated skin

The removed skin tissue (about 100 mg) was washed in phosphate-buffered saline (PBS, pH 7.0) and cut into small pieces, and then tissue protein extraction reagent (T-PER) containing protease inhibitor (Pierce Co., Rockford, IL) (2 ml) was added, and the mixture was homogenized. The skin homogenate was centrifuged at 2000 ×*g* for 10 min at 4 °C. The VEGF content of the supernatant was determined using a VEGF ELSA kit (R&D Systems, Minneapolis, MN). The MMP-2 (active and inactive forms) and MMP-9 (active and inactive form) in the supernatant were separated by electrophoresis on a 7.5% sodium dodecyl sulfate (SDS) polyacrylamide gel containing 0.1% gelatin under non-reducing conditions. The gel was then washed with 50 mM Tris–HCl (pH 7.5) containing 100 mM NaCl and 2.5% Triton X-100 for 1.5 h, and incubated in 50 mM Tris–HCl containing 10 mM $CaCl_2$ and 10 μM $ZnCl_2$ at 37 °C for 20 h. It was stained with 0.25% Coomassie Brilliant Blue 250, and the unstained gelatin-degraded zone was quantified using NIH Image J 1.36.

2.6. Histological examination of UVB-irradiated skin

Dorsal skin samples (about 3 cm^2) were fixed in 10% buffered formalin for at least 24 h, progressively dehydrated in solutions containing an increasing percentage of ethanol (70, 80, 95, and 100%, v/v), cleared in Histoclear (AS-ONE, Tokyo, Japan), embedded in paraffin under vacuum, sectioned 5-μm-thick, deparaffinized, and stained with hematoxylin–eosin (HE) and Azan stain. After the same cross-sections were selected from three plates per sample, four different microscopic fields (40×, 100×, or 200×) per plate were photographed. The thickness of the epidermis and dermis was measured using a Digimatic caliper.

2.7. Cell culture, and western blotting for COX-2, HIF-1α, and NF-κB/p65 protein (in vitro)

The human keratinocyte cell line HaCaT was obtained from the Department of Dermatology, Ehime University Graduate School of Medicine, and maintained in DMEM supplemented with 10% FBS,

penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin B (0.25 μg/ml). HaCaT cells (3×10^5) were seeded in 100-mm culture dishes. After subconfluency was reached, the medium was changed to serum-free medium and the cells cultured for 24 h. The cells were washed with phosphate-buffered saline (PBS, pH 7.0) and irradiated with UVB at 5 mJ/cm^2. After the irradiation, the cells were cultured in serum-free medium with the indicated amounts of wogonin and baicalein for 12 h. They were washed with PBS, and lysed with cell lysis buffer [20 mM Tri–HCl (pH 7.5) containing 150 mM NaCl, 1 mM ethylemediaminetetraacetic acid (EDTA), 1 mM ethylene glycol-*bis* (2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 1% Triton, 2.5 mM sodium pyrophosphatase, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupetin, and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. After centrifugation at 14,000×*g* for 10 min at 4 °C, the supernatant was used to measure COX-2 protein levels. Samples (40 μg protein) were subjected to electrophoresis in a 7.5% polyacrylamide gel, and used for a western blot analysis with rabbit polyclonal anti-COX-2 and mouse monoclonal anti-β-actin antibodies. The nuclear fraction of the cells was extracted for the measurement of HIF-1α protein levels. Briefly, the cells were washed with ice-cold PBS and pelleted. They were further rinsed with detergent-free buffer A [10 mM Hepes buffer (pH 7.9) containing 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiotheritol (DTT), 1 mM Na_3VO_4, 10 mM β-glycerophosphate,, 5 μg/ml aprotinin, 1 μg/ml leupeptin, 2 μg/ml pepstatin A, and 1 mM PMSF], then were resuspended in buffer A containing 0.1% NP-40 and allowed to swell for 10 min. The cells were scraped and passed 10 times through a 25-guage needle to disrupt cell membranes. After centrifugation at 1500×*g* for 5 min at 4 °C, the nuclear pellets were resuspended in buffer B [20 mM Hepes buffer (pH 7.9) containing 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 25% glycerol, 1 mM Na_3VO_4, 10 mM β-glycerophosphate, 5 μg/ml aprotinin, 1 μg/ml leupeptin, 2 μg/ml pepstatin A, and 1 mM PMSF] and incubated on ice for 30 min. After centrifugation at 15,000×*g* for 10 min at 4 °C, the supernatant (30 μg protein) was subjected to electrophoresis in a 5% polyacrylamide gel, and used for western blotting with mouse monoclonal anti-HIF-1α and mouse monoclonal anti-β-actin antibodies. The measurement of NF-κB/p65 protein in nuclear fraction was performed as follows; the subconfluent HaCaT cells were cultured in medium supplemented with 10% FBA and the indicated amount of baicalein and wogonin for 12 h. The cells were washed with PBS and irradiated with UVB at 50 mJ/cm^2. After irradiation, the cells were further cultured in FBS-containing medium with baicalein or wogonin for 3 h, and then the preparation of nuclear fraction were performed by the same methods described earlier. After centrifugation, the supernatant (40 μg protein) was subjected to electrophoresis in a 7.5% polyacrylamide gel, and sued for western blotting with rabbit polyclonal anti-NF-κB/p65 antibody.

2.8. Statistical analysis

All values are expressed as the mean ± S.E.M. Data were analyzed by one-way ANOVA or repeated-measures ANOVA. When the F-test was significant, means were compared using the Tukey–Kramer or Dunnet test with Stat View (SAS Institute Inc., Tokyo, Japan). Differences were considered significant at $P<0.05$.

3. Results

3.1. Effects of baicalein and wogonin isolated from S. baicalensis roots on skin and ear thickness in acute UVB-irradiated hairless mice

Skin and ear thickness increased significantly 2 to 7 days after UVB irradiation, compared to that in unexposed mice (normal) (Fig. 2). The increase was significantly inhibited by baicalein (10 and 50 mg/kg, twice daily) and wogonin (10 and 50 mg/kg, twice daily) compared with the control (vehicle-treated UVB-irradiated mice) (Fig. 2A). Baicalein (10 and 50 mg/kg) and wogonin (50 mg/kg) significantly inhibited this increase compared with the control (Fig. 2B).

3.2. Effects of baicalein and wogonin on the thickness of the epidermis and extracellular matrix(ECM) of the dermis in acute UVB-irradiated hairless mice

The thickness of the epidermis and ECM in the dermis was increased by acute UVB irradiation. Baicalein (10 and 50 mg/kg) and wogonin (50 mg/kg) significantly inhibited thickening of the epidermis (Table 1 and Fig. 3). The thickening of the ECM in the dermis was also inhibited by the oral administration of baicalein (10 and 50 mg/kg), and wogonin (10 and 50 mg/kg).

3.3. Effects of baicalein and wogonin on the expression of MMP-2, MMP-9, and VEGF in acute UVB-irradiated mice

The expression of pro-MMP-2 (inactive form), MMP-2 (active form), pro-MMP-9 (inactive form), and MMP-9 (active form) was significantly greater in acute UVB-irradiated mice (control) than unexposed mice (normal) (Fig. 4). The increases in pro-MMP-9 and MMP-9 in the control were significantly inhibited by the oral administration of baicalein and wogonin at doses of 10 and 50 mg/kg, respectively (Fig. 4). The increase in pro-MMP-2 in the control was inhibited by baicalein (10 mg/kg), but not baicalein (50 mg/kg), or wogonin (10 and 50 mg/kg), (Fig. 4). Baicalein, and wogonin had no effect on the increase in MMP-2 induced by UVB irradiation (Fig. 4).

The VEGF content of the skin was also significantly increased by the acute irradiation. Baicalein (10 and 50 mg/kg), and wogonin (50 mg/kg) significantly inhibited this increase (Table 2).

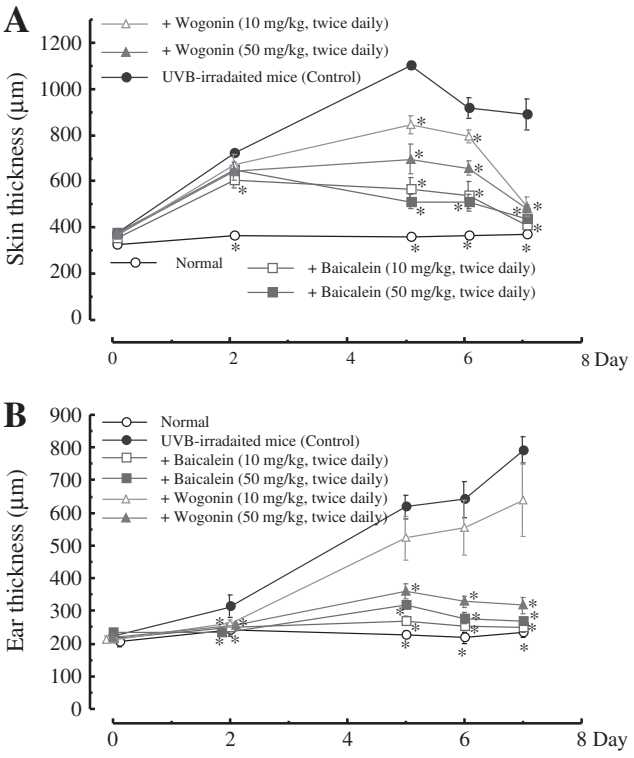

Fig. 2. Effects of baicalein and wogonin on skin and ear thickness in acute UVB-irradiated mice. Values are the mean ± S.E.M. for 6 mice. *Significantly different from UVB-irradiated mice (control), $P<0.05$.

Table 1
Effects of baicalein and wogonin isolated from *Scutellaria baicalensis* roots on the thickness of the epidermis and extracellular matrix (ECM) of the dermis in acute UVB-irradiated hairless mice.

	Epidermis (μm)[a]	ECM (μm)in dermis[a]
Normal mice	27.86 ± 2.68[b]	214.04 ± 17.70[b]
Vehicle-treated UVB-irradiated mice (control)	141.32 ± 18.78	528.36 ± 37.25
+ Baicalein (10 mg/kg, twice daily)	42.28 ± 3.63[b]	321.21 ± 39.35[b]
(50 mg/kg, twice daily)	57.27 ± 11.38[b]	297.11 ± 26.55[b]
+ Wogonin (10 mg/kg, twice daily)	158.35 ± 13.43	398.88 ± 45.84[b]
(50 mg/kg, twice daily)	83.01 ± 16.28[b]	398.06 ± 15.09[b]

[a] Values are means ± S.E.M. for 6 mice.
[b] Significantly different from UVB-irradiated mice (control), $P<0.05$.

3.4. Effects of baicalein and wogonin on the diameter and length of blood vessels in the skin of acute UVB-irradiated mice

The diameter and length of blood vessels were significantly increased by acute UVB irradiation. Baicalein (10 and 50 mg/kg) inhibited the increase in the diameter of blood vessels, but wogonin did not. The increase in vessel length was inhibited by the oral administration of baicalein (10 and 50 mg/kg), and wogonin (10 and 50 mg/kg) (Fig. 5, and Table 3).

3.5. Effects of baicalein, and wogonin on COX-2, HIF-1α and NF-kB/p65 expression in UVB-irradiated HaCaT cells (in vitro)

The levels of COX-2 expression were stimulated by UVB radiation. Baicalein and wogonin inhibited this increase at concentrations of 50 and 100 μM in UVB-irradiated HaCat cells (Fig. 6A). The levels of nuclear HIF-1α expression were stimulated by UVB radiation. Baicalein stimulated HIF-1α expression at 10, 50 and 100 μM in

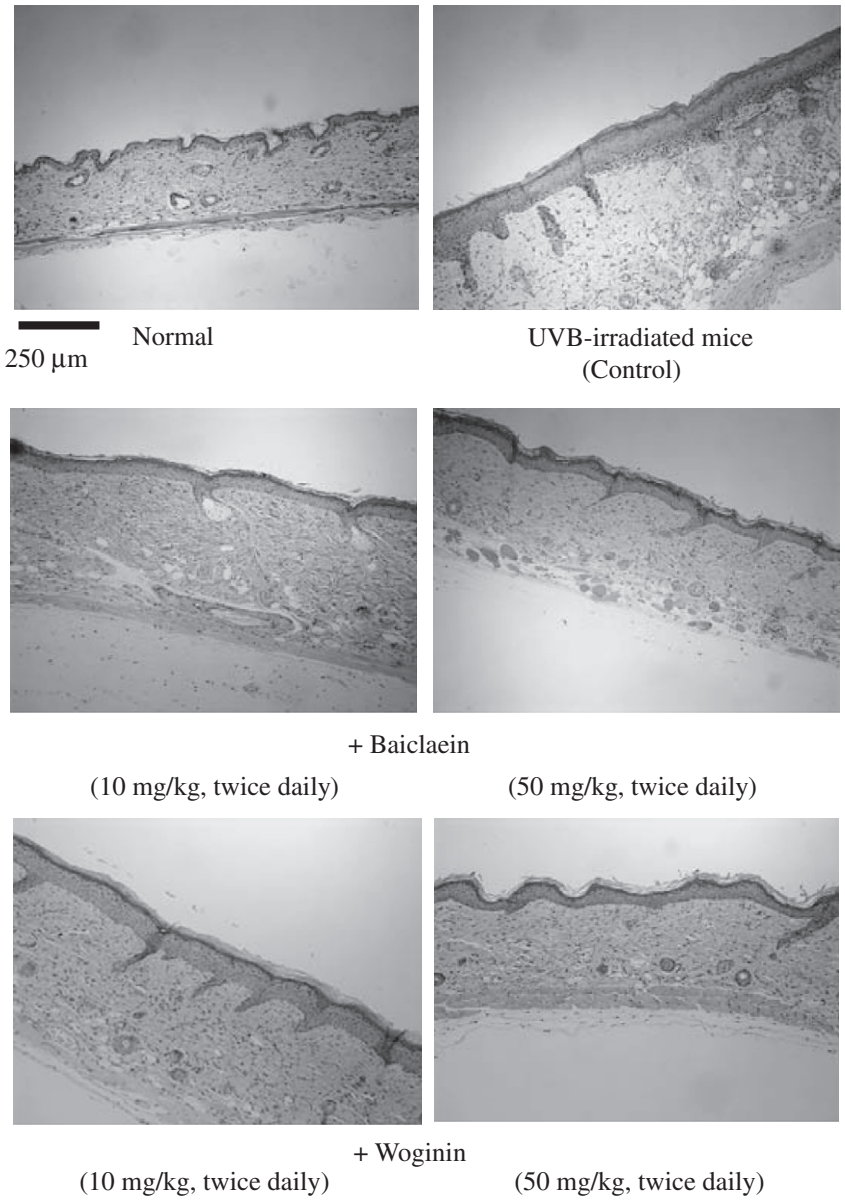

Fig. 3. Light micrographs of cells stained with hematoxylin–eosin (HE) to show the thickness of the epidermis and dermis in normal mice, vehicle-treated acute UVB-irradiated mice (control), and baicalein-treated and wogonin-treated UVB-irradiated mice.

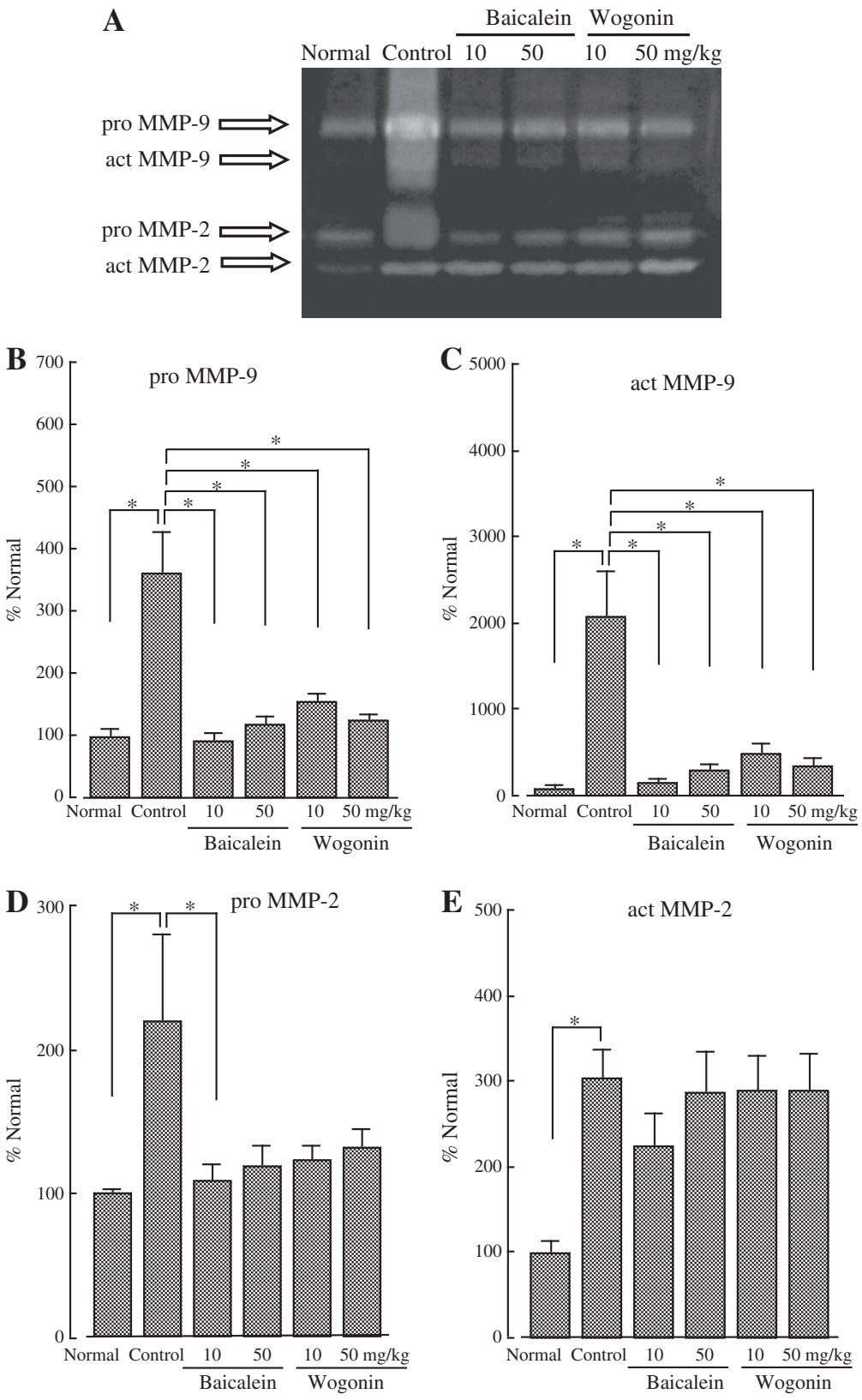

Fig. 4. Effects of baicalein and wogonin on pro-MMP-9, MMP-9, pro-MMP-2, and MMP-2 expression in the skin of acute UVB-irradiated mice. Values are the mean ± S.E.M. for 6 mice. *Significantly different from UVB-irradiated mice (control), $P<0.05$.

both non-UVB- and UVB-irradiated HaCaT cells, whereas wogonin suppressed it at 10, 50 and 100 μM in UVB-irradiated HaCaT cells (Fig. 6B). The levels of NF-κB/p65 expression in nuclear fraction were stimulated by UVB radiation. Baicalein inhibited the increase in nuclear NF-kB/p65 expression at concentration of 50 and 100 μM in UVB-irradiated HaCaT cells, but wogonin had no effect (Fig. 6C).

Table 2
Effects of baicalein and wogonin on VEGF levels in the skin of acute UVB-irradiated hairless mice.

	VEGF (pg/mg protein)[a]	% of control
Normal mice	11.72 ± 0.84[b]	9.37
Vehicle-treated UVB-irradiated mice (control)	125.14 ± 25.59	100
+ Baicalein (10 mg/kg, twice daily)	34.52 ± 3.54[b]	27.6
(50 mg/kg, twice daily)	31.12 ± 2.05[b]	24.9
+ Wogonin (10 mg/kg, twice daily)	98.79 ± 22.45	78.9
(50 mg/kg, twice daily)	48.25 ± 9.25[b]	38.6

[a] Values are means ± S.E.M. for 6 mice.
[b] Significantly different from UVB-irradiated mice (control), $P<0.05$.

Table 3
Effects of baicalein and wogonin on the diameter and length of blood vessels in the skin of acute UVB-irradiated hairless mice.

	Diameter (μm)[a]	Length (mm/cm^2 area)[a]
Normal mice	248.60 ± 14.58[b]	70.38 ± 1.92[b]
Vehicle-treated UVB-irradiated mice (control)	437.60 ± 38.27	109.73 ± 8.87
+ Baicalein (10 mg/kg, twice daily)	301.23 ± 28.92[b]	88.02 ± 4.51[b]
(50 mg/kg, twice daily)	236.47 ± 28.88[b]	87.42 ± 5.60[b]
+ Wogonin (10 mg/kg, twice daily)	416.39 ± 18.66	81.51 ± 5.58[b]
(50 mg/kg, twice daily)	357.46 ± 29.04	87.04 ± 7.12[b]

[a] Values are means ± S.E.M. for 6 mice.
[b] Significantly different from UVB-irradiated mice (control), $P<0.05$.

4. Discussion

Exposure to solar UV radiation has serious effects on the structure and function of skin. The number of cases of non-melanoma skin cancers is estimated at over 700,000 and expected to rise as more UV radiation reaches the earth because of depletion of the ozone layer (Boring et al., 1993; Miller and Weinstock, 1994; O'Shaughnessy et al., 2002). The symptoms of cutaneous aging, including wrinkles and pigmentation, develop earlier in sun-exposed skin than in unexposed skin, a phenomenon referred to as photoaging. UVB radiation is an important environmental factor because of its hazardous effects, which include the generation of skin cancer (de Gruijl et al., 1993),

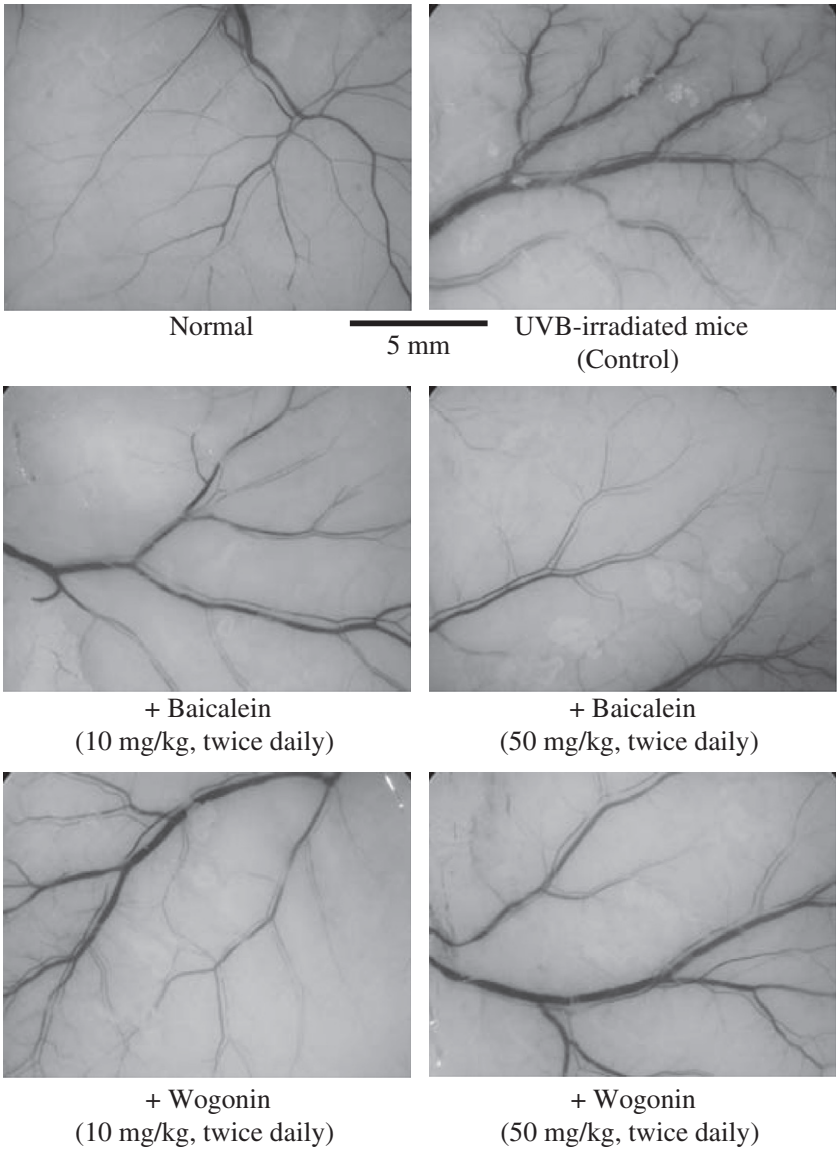

Fig. 5. Photographs showing skin blood vessels in normal mice, vehicle-treated acute UVB-irradiated mice (control), and baicalein-treated and wogonin-treated UVB-irradiated mice.

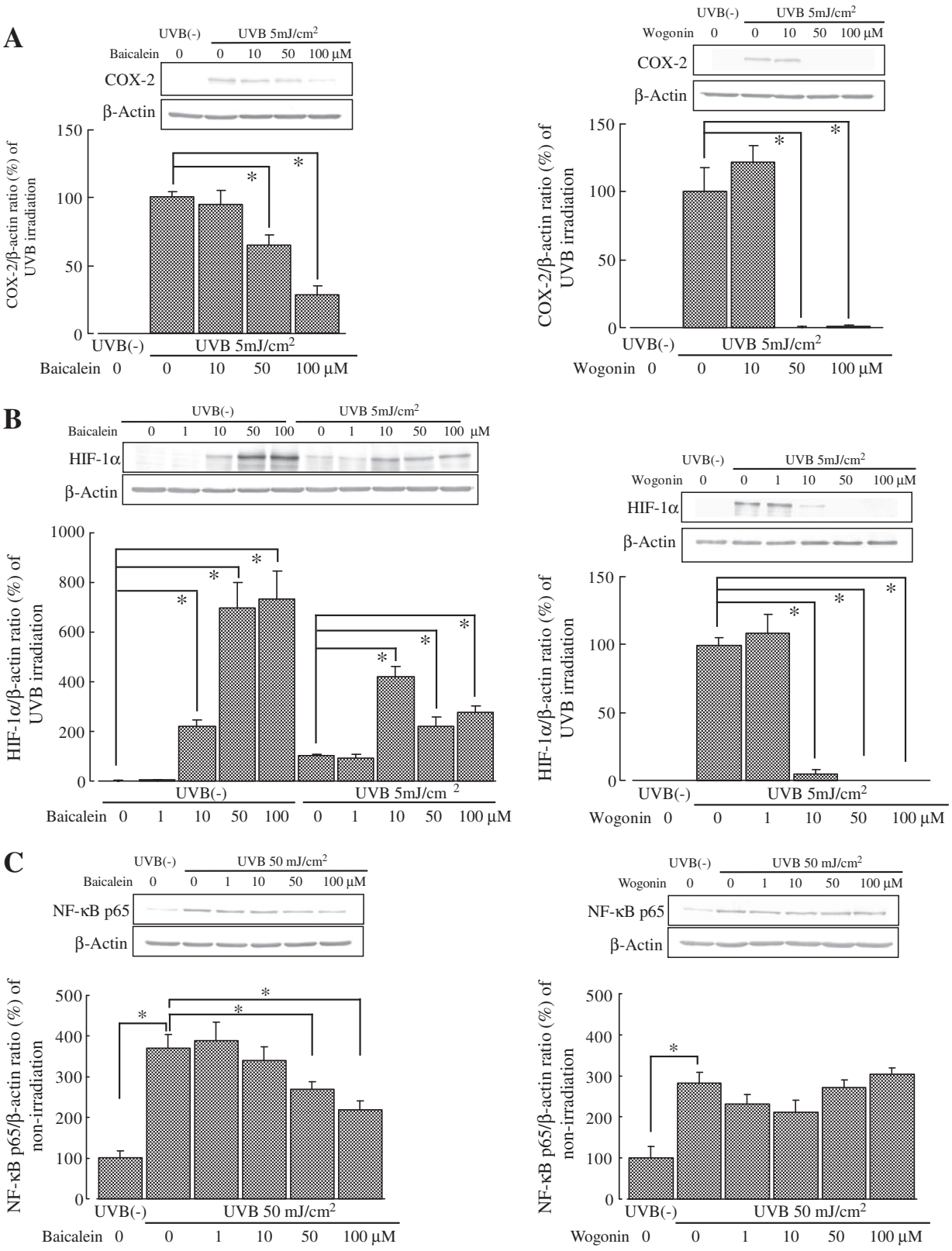

Fig. 6. Effects of baicalein and wogonin, on COX-2, HIF-1α and NF-kB/p65 expression in HaCaT cells (*in vitro*). Values are the mean ± S.E.M. for 4 experiments. *Significantly different from UVB-irradiated mice (control), $P<0.05$.

suppression of the immune system (Inomata et al., 2003), skin inflammation and edema (Afaq et al., 2003), and premature skin aging (Fisher et al., 1997). Skin alterations observed after UVB irradiation include erythema, vascular hyper-permeability, dilation of dermal blood vessels, and epidermal hyperplasia (Berton et al., 1997; Cox et al., 1992; Kripke, 1994; Pearse et al., 1987).

Baicalein and wogonin reduced the increase in skin thickness and ear thickness induced by acute UVB irradiation. They also

inhibited the increase in diameter and length/cm^2 skin area of blood vessels. Thus, baicalein and wogonin prevented inflammation of the skin induced by exposure to UVB irradiation. Furthermore, they reduced the increase in the levels of MMP-9 and VEGF expression in irradiated skin. To clarify the mechanisms of the inhibitory effects of baicalein, and wogonin on skin inflammation induced by acute UVB irradiation, we examined the two's effects on the levels of COX-2, nuclear HIF-1α and NF-kB/p65 expression induced by exposure to irradiation in the human keratinocyte cell line HaCaT.

Hwang et al. (2008) reported that baicalein suppressed the hypoxia-induced production and activation of HIF-1α as well as the expression of hypoxia-responsive genes by inhibiting reactive oxygen species and the PI3-kinase/Akt pathway in BV2 murine microglial cells. Liu et al. (2003) reported that baicalein had a potent inhibitory effect on angiogenesis through the inhibition of MMP-2 expression in human umbilical vein endothelial cells. However, baicalein and wognin had no effect on the increase in skin MMP-2 expression induced by acute UVB irradiation. Furthermore, Cho et al. (2008) reported that baicalein induced the expression of a functional HIF-1α and angiogenesis. Thus, there are perplexing contradictions in the reported effects of baicalein on HIF-1α expression or angiogenesis. In this study, the expression of HIF-1α was increased by UV irradiation in HaCaT cells, and it was stimulated by baicalein in UVB-irradiated and non-irradiated HaCaT cells. Triantafyllou et al. (2008) reported that flavonoids including quercetin, luteolin, and baiclein induced HIF-1α expression. Cho et al. (2009) reported that UVB was found to suppress HIF-1α acutely in cultured keratinocytes and mouse skin, and suggested that the deregulation of HIF-1α was associated with UVB-induced hyperplasia of the epidermis. Therefore, the inhibitory effects of baicalein on UVB-irradiated hyperplasia in skin epidermis may be due to the stimulation of nuclear HIF-1α expression. Further study is needed to clarify the mechanism of baicalein on HIF-1α expression after the irradiation in skin damage. The levels of COX-2 and nuclear NF-kB/p65 were increased by UVB irradiation in HaCaT cells. Baicalein inhibited these increases. Hsieh et al. (2007) reported that baicalein inhibited IL-1β- and TNF-α-induced inflammatory cytokine production from human mast cells thorough the regulation of the NF-κB pathway. Furthermore, Wu et al. (2010) reported that baicalein inhibited the NF-κB and apoptosis though the cellular FLICE inhibitory protein and mitogen activated protein kinase in D-galcatosamine/lipopolysacchride-induced liver injury of mice. Therefore, the inhibitory effects of baicalein on acute irradiation-induced skin inflammation including skin edema may be due to the suppression of increases in the levels of MMP-9 and VEGF through a reduction in COX-2 expression and NF-κB activation. Wogonin inhibited the expression of COX-2 and HIF-1α in UVB-treated HaCaT cells, but it had no effect on the expression of NF-κB/p65. Jung et al. (2003) reported that COX-2 inhibitors attenuated IL-1β mediated HIF-1α induction, and that prostaglandin E_2 (COX-2 product) induced HIF-1α protein. Consequently, the inhibitory effects of wogonin on irradiation-induced skin inflammation may be due to the inhibition of increases in the levels of MMP-9 and VEGF through the inhibition of COX-2 and HIF-1α expression. Further studies are needed to clarify the mechanistic difference of inhibitory effects of baicalein and wogonin on acute UVB irradiation-induced skin damage.

Experiments are now in progress to study the inhibitory effects of baicalein, and wogonin on chronic UVB irradiation-induced carcinogenesis in hairless mice.

Acknowledgements

This work was supported in part by a Grant-in aid for Scientific Research (C) (No. 20590700 to Yoshiyuki Kimura) from the Ministry of Education, Culture Sports, Science and Technology.

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