Biochemical and Biophysical Research Communications 438 (2013) 375–381

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

[8]-Gingerol inhibits melanogenesis in murine melanoma cells through down-regulation of the MAPK and PKA signal pathways

Huey-Chun Huang [a], Yin-Chun Chou [b], Chia-Yin Wu [b], Tsong-Min Chang [b,*]

[a] Department of Medical Laboratory Science and Biotechnology, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan, ROC
[b] Department of Applied Cosmetology and Master Program of Cosmetic Science, HungKuang University, No. 1018, Sec. 6, Taiwan Boulevard, Shalu District, Taichung City 43302, Taiwan, ROC

ARTICLE INFO

Article history:
Received 6 July 2013
Available online 25 July 2013

Keywords:
[8]-Gingerol
Melanogenesis
Tyrosinase
Melanin

ABSTRACT

[8]-Gingerol is an active component of Zinger and shows several pharmacological activities, such as antipyretic and anti-inflammation characteristics. To identify a potential skin-whitening agent, the inhibitory effects of [8]-gingerol on melanogenesis and its mechanism of action were investigated. In the present study, the effects of [8]-gingerol on mushroom tyrosinase, tyrosinase activity and melanin content were determined spectrophotometrically; the expression of melanogenesis-related proteins in B16F10 and B16F1 melanoma cells were determined by Western blotting. Furthermore, the possible signaling pathways involved in [8]-gingerol-mediated depigmentation were also investigated using specific inhibitors. The results revealed that [8]-gingerol (5–100 μM) effectively suppressed intracellular tyrosinase activity and decreased the amount of melanin in B16F10 and B16F1 cells. In addition, [8]-gingerol also effectively decreased intracellular reactive species (RS) and reactive oxygen species (ROS) levels at the same dose range. Our results indicated that [8]-gingerol inhibited melanogenesis in B16F10 and B16F1 cells by down-regulation of both mitogen-activated protein kinases (MAPK) and protein kinase A (PKA) signaling pathways or through its antioxidant properties. Hence, [8]-gingerol could be used as an effective skin-whitening agent.

1. Introduction

Melanin is responsible for skin color and plays an important role in protecting the skin against ultraviolet light injury. It has been reported that overproduction and accumulation of melanin causes several skin disorders, including melasma, freckles, age spots and other hyperpigmentation syndromes [1]. Tyrosinase is the rate-limiting enzyme in the first two steps of melanogenesis in which L-tyrosine is hydroxylated to L-DOPA (L-dihydroxyphenylalanine), and L-DOPA is further oxidized into the corresponding *o*-quinone [2]. Moreover, the microphthalmia-associated transcription factors (MITF) and other enzymes, such as tyrosinase related protein-1 (TRP-1) and tyrosinase related protein-2 (TRP-2), have been reported to contribute to the production of melanin [3–5]. The melanocortin 1 receptor (MC1R) also plays an important role in MSH-induced pigmentation [6]. Recently, inhibitors of melanogenesis have been increasingly applied in skin care products for the treatment or prevention of abnormal hyperpigmentation [7]. Furthermore, it is reported that melanogenesis produces hydrogen peroxide (H_2O_2) and other ROS and RS, which lead to the generation of high-grade oxidative stress in melanocytes. Hence, free radical scavengers play an important role in the regulation of melanogenesis. It has been established that ROS scavengers and inhibitors of ROS generation may inhibit UV-induced melanogenesis [8]. Therefore, antioxidants or compounds with redox properties could also be used to inhibit or delay hyperpigmentation [2]. Furthermore, antioxidants such as ascorbic derivatives and reduced glutathione (GSH) have been applied as inhibitory agents of melanogenesis [9,10].

It has been reported that numerous phenolic compounds show antioxidant activity, and many naturally occurring inhibitors of melanogenesis contain a phenol structure [11–13]. We recently reported that [6]-gingerol inhibited melanin synthesis in B16F10 melanoma cells through the activation of the PI3K signaling pathway [14]. Another active phenolic ingredient of *Zingiber officinale* roscope (Zingiberaceae), [8]-gingerol (Fig. 1A), is reported to possess various pharmacological activities, including immunosuppressive activity [15], cardiotonic effects [16] and anti-inflammatory capacities [17]. However, the inhibitory action of [8]-gingerol on melanogenesis has never been explored. In the present study, we

* Corresponding author. Fax: +886 4 26315843.
E-mail addresses: lchuang@mail.cmu.edu.tw (H.-C. Huang), friesing@hotmail.com (Y.-C. Chou), michellewu27@gmail.com (C.-Y. Wu), ctm@sunrise.hk.edu.tw (T.-M. Chang).

0006-291X
http://dx.doi.org/10.1016/j.bbrc.2013.07.079

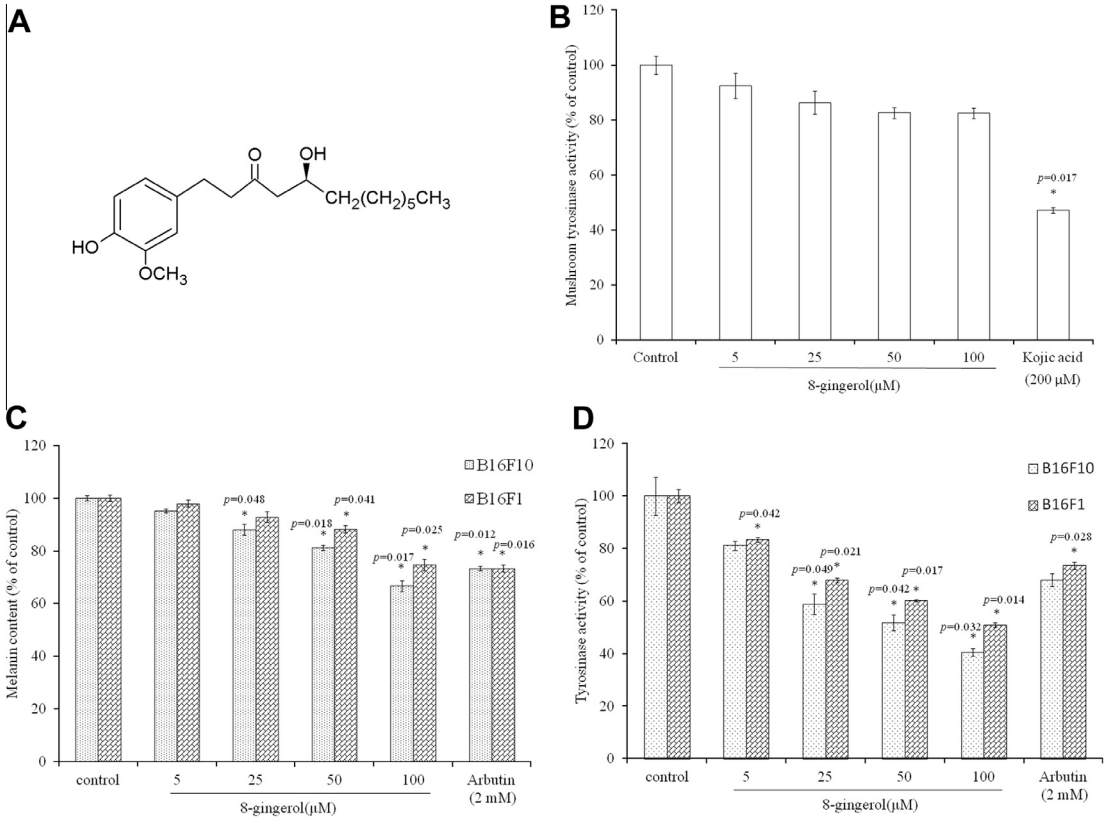

Fig. 1. The inhibitory effects of [8]-gingerol on melanogenesis. (A) The chemical structure of [8]-gingerol. (B) The effects of [8]-gingerol on mushroom tyrosinase activity. (C) The effects of [8]-gingerol on melanin content in B16F10 and B16F1 cells. (D) The effects of [8]-gingerol on tyrosinase activity in B16F10 and B16F1 cells. The results are presented as percentages of the control, and the data are presented as the mean ± S.D. of three separate experiments. The values are significantly different compared with the control. $^{*}p < 0.05$.

attempted to investigate the effects of [8]-gingerol on mushroom tyrosinase activity, the expression of melanogenesis-related proteins, and melanin content in B16F10 and B16F1 melanoma cells. Moreover, we examined the inhibitory effect and associated antioxidant property of [8]-gingerol on melanogenesis by assessing its free radical scavenging activities. Furthermore, to examine the possible signaling pathways involving [8]-gingerol, we also studied the changes of MAPKs and protein kinase A (PKA) signaling inhibited by [8]-gingerol.

2. Materials and methods

2.1. Chemicals and reagents

The [8]-gingerol was purchased from WAKO (Japan). The antibodies were from Santa Cruz Biotech (Santa Cruz, CA, USA), and the ECL reagent was from Millipore (MA, USA). Protein kinase regulators, including 3-isobutyl-1-methyl-xanthine (IBMX), SB203580 (p38 MAPK-inhibitor), SP600125 (c-Jun N-terminal kinase inhibitor; JNK inhibitor) and PD98059 (MEK 1/2-inhibitor) were from Tocris (Ellisville, Missouri, USA). All of the other chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Cell culture

The B16F10 cells (ATCC CRL-6475, BCRC60031) and B16F1 cells (ATCC CRL-6323, BCRC60030) were obtained from the Bioresource Collection and Research Center (BCRC), Taiwan. The B16F10, B16F1 and HaCaT cells [18] were maintained in DMEM (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum and 1% antibiotics at 37 °C, 5% CO_2 in a humidified incubator.

2.3. Cell viability assay

The assay was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [19]. The cells were exposed to various concentrations of [8]-gingerol for 24 h, and the MTT solution was then added to the wells. The insoluble derivative of MTT produced by intracellular dehydrogenase was solubilized with ethanol–DMSO (1:1 mixture solution). The absorbance of the wells at 570 nm was read using a microplate reader.

2.4. Assay of mushroom tyrosinase activity

The enzyme inhibition experiments were conducted as previously described [20]. Briefly, 10 μL of the aqueous solution of mushroom tyrosinase (200 units) was added to a 96-well microplate, for a total volume of a 200 μL mixture containing 5 mM L-DOPA, which was dissolved in 50 mM phosphate buffered saline (PBS) (pH 6.8). The assay mixture was incubated at 37 °C for 30 min and the absorbance of dopachrome was measured at 490 nm.

2.5. Measurement of melanin content

The intracellular melanin content was measured as described by Tsuboi et al. [21]. The cells were treated with α-MSH (100 nM) for 24 h, and the melanin content was then determined after treatment with either [8]-gingerol (5–100 μM) or arbutin (2 mM) for additional 24 h. After treatment, the cell pellets containing a known number of cells were solubilized in 1 N NaOH at 60 °C for 60 min. The melanin content was assayed at 405 nm.

2.6. Assay of intracellular tyrosinase activity

The cellular tyrosinase activity was determined as described previously [22]. The cells were treated with α-MSH (100 nM) for 24 h and then with [8]-gingerol (5–100 μM) or arbutin (2 mM) for 24 h. After treatments, the cell extracts (100 μL) were mixed with freshly prepared L-DOPA solution (0.1% in PBS) and incubated at 37 °C, the absorbance at 490 nm was measured.

2.7. Western blotting assay

The cells were treated with [8]-gingerol (5–100 μM) or kojic acid (200 μM), lysed in PBS containing proteinase inhibitor at 4 °C for 20 min. Proteins (50 μg) were resolved by SDS–polyacrylamide gel electrophoresis and electrophoretically transferred to a polyvinylidene fluoride (PVDF) filter. The nylon filter was blocked for 1 h in 5% fat-free milk in PBST buffer (PBS with 0.05% Tween-20). After a brief wash, the filter was incubated overnight at 4 °C with several antibodies; these antibodies included anti-MITF (1:1000), anti-TRP1 (1:6000), anti-TRP2 (1:1000), anti-MC1R (1:500), anti-GAPDH (1:1500), anti-tyrosinase (1:2000), anti-p-p38 (1:500), anti-p38 (1:500), anti-p-JNK (1:500), anti-JNK (1:500), anti-p-ERK (1:500), anti-ERK (1:500), anti-p-CERB (1:500), and anti-CERB (1:200). Following incubation, the filter was extensively washed in PBST buffer. Subsequent incubation with goat anti-mouse antibody (1:10,000) conjugated with horseradish peroxidase was conducted at room temperature for 2 h. The blot was visualized using an ECL reagent. The relative amounts of expressed proteins compared to total GAPDH were analyzed using Multi Gauge 3.0 software (Fuji, Tokyo).

2.8. Protein kinase regulators assay

The cells were treated with α-MSH (100 nM) for 24 h followed by a 1 h addition of 10 μM of different protein kinase regulators, including IBMX, SB203580, SP600125 and PD98059. After these treatments, 100 μM of [8]-gingerol and 10 μM of the above kinase regulators were added to the cells and incubated for an additional 23 h. The melanin contents were assayed as described above.

2.9. Determination of cellular ROS and RS level

The cells were cultured in 24-well plates and treated with [8]-gingerol (5–100 μM) for 24 h. The cells were then incubated with 24 mM H_2O_2 at 37 °C for 30 min. After incubation, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) was added to the wells and cultured for 30 min. The fluorescence intensities of DCF were measured at an excitation wavelength of 504 nm and an emission wavelength of 524 nm [23] using a Fluoroskan Ascent fluorescent reader (Thermo Scientific, Vantaa, Finland). The data were analyzed using Ascent software (Thermo Scientific, Vantaa, Finland). Reactive species (RS) were evaluated in the culture supernatant [24]. Twenty-five millimolar of DCFH-DA was added to the incubation media, and changes in fluorescence were estimated at an excitation wavelength of 486 nm and an emission wavelength of 530 nm for 30 min.

2.10. Statistical analysis

Statistical analysis of the experimental data was performed using the two-tailed *t*-test including the *p*-values. All of the differences were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Inhibitory effects of [8]-gingerol on melanogenesis

The results shown in Fig. 1B, reveal that the remaining mushroom tyrosinase activity was 92.48 ± 4.55%, 86.34 ± 4.25%, 82.66 ± 1.95% and 82.54 ± 1.95% of the control for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively. In addition, the tyrosinase activity was also inhibited by kojic acid (200 μM), and the remaining enzyme activity was 47.06 ± 0.98% of control ($p < 0.05$) (Fig. 1 B). Thus, [8]-gingerol may be an inhibitor of mushroom tyrosinase. The results in Fig. 1C, indicate that [8]-gingerol significantly decreased the melanin content in B16F10 and B16F1 cells. After treatment, the melanin content in B16F10 cells was 95.3 ± 0.74%, 88.11 ± 1.97%, 81.27 ± 0.94% and 66.74 ± 2.05% for the 5, 25, 50 and 100 μM [8]-gingerol treatments, respectively. The remaining intracellular melanin content was 77.35 ± 0.90% of the control for arbutin (2 mM) ($p < 0.05$). Meanwhile, the melanin content in B16F1 cells was 98.02 ± 1.43%, 92.88 ± 2.08%, 88.26 ± 1.48% and 74.8 ± 2.15% for the 5, 25, 50 and 100 μM [8]-gingerol treatments, respectively. The remaining intracellular melanin content was 73.35 ± 1.25% of the control for arbutin (2 mM) ($p < 0.05$). The results indicate that 100 μM of [8]-gingerol has stronger effects than arbutin. The remaining B16F10 intracellular tyrosinase activity was 81.19 ± 1.7%, 58.87 ± 3.77%, 51.7 ± 2.99% and 40.53 ± 1.55% for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively. The intracellular tyrosinase activity was 68.03 ± 2.4% after the cells were treated with arbutin (2 mM) ($p < 0.05$). In addition, the remaining B16F1 intracellular tyrosinase activity was 83.41 ± 0.85%, 67.96 ± 0.77%, 60.19 ± 0.39% and 50.92 ± 0.9% for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively. The intracellular tyrosinase activity was 73.45 ± 1.41% after the cells were treated with arbutin (2 mM) ($p < 0.05$) (Fig. 1D). The results indicate that [8]-gingerol exhibited a more potent inhibitory effect on α-MSH-induced tyrosinase activity in B16F10 and B16F1 cells than did arbutin.

3.2. [8]-Gingerol inhibited melanogenesis-related protein expression in B16F10 and B16F1 cells

The expression levels of melanogenesis-related proteins were examined using Western blots (Fig. 2A and E). The results indicate that 5–100 μM of [8]-gingerol treatment led to a significantly reduced level of MC1R, and 100 μM of [8]-gingerol significantly decreased the expression of TRP-2 (Fig. 2B and F). Furthermore, the protein contents of MITF, TRP-1 and tyrosinase were slightly changed after [8]-gingerol treatment.

3.3. [8]-Gingerol down-regulated the cAMP signaling pathway

The PKA signaling pathway is associated with regulating melanogenesis. The impacts of [8]-gingerol on the expression and phosphorylation of CREB were examined using Western blots (Fig. 2C and G). The results show that [8]-gingerol decreased the expression of p-CREB; the ratios of p-CREB/CREB in B16F10 cells were 1.13, 0.9, 0.88 and 0.87 for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively (Fig. 2D). Also, the ratios of p-CREB/CREB in B16F1 cells were 1.12, 0.92, 0.86 and 0.8 for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively (Fig. 2H). The application of [8]-gingerol in IBMX-treated B16F10 cells significantly decreased the cellular melanin content. The results indicate that cAMP-mediated PKA signaling was affected by [8]-gingerol (Fig. 3A).

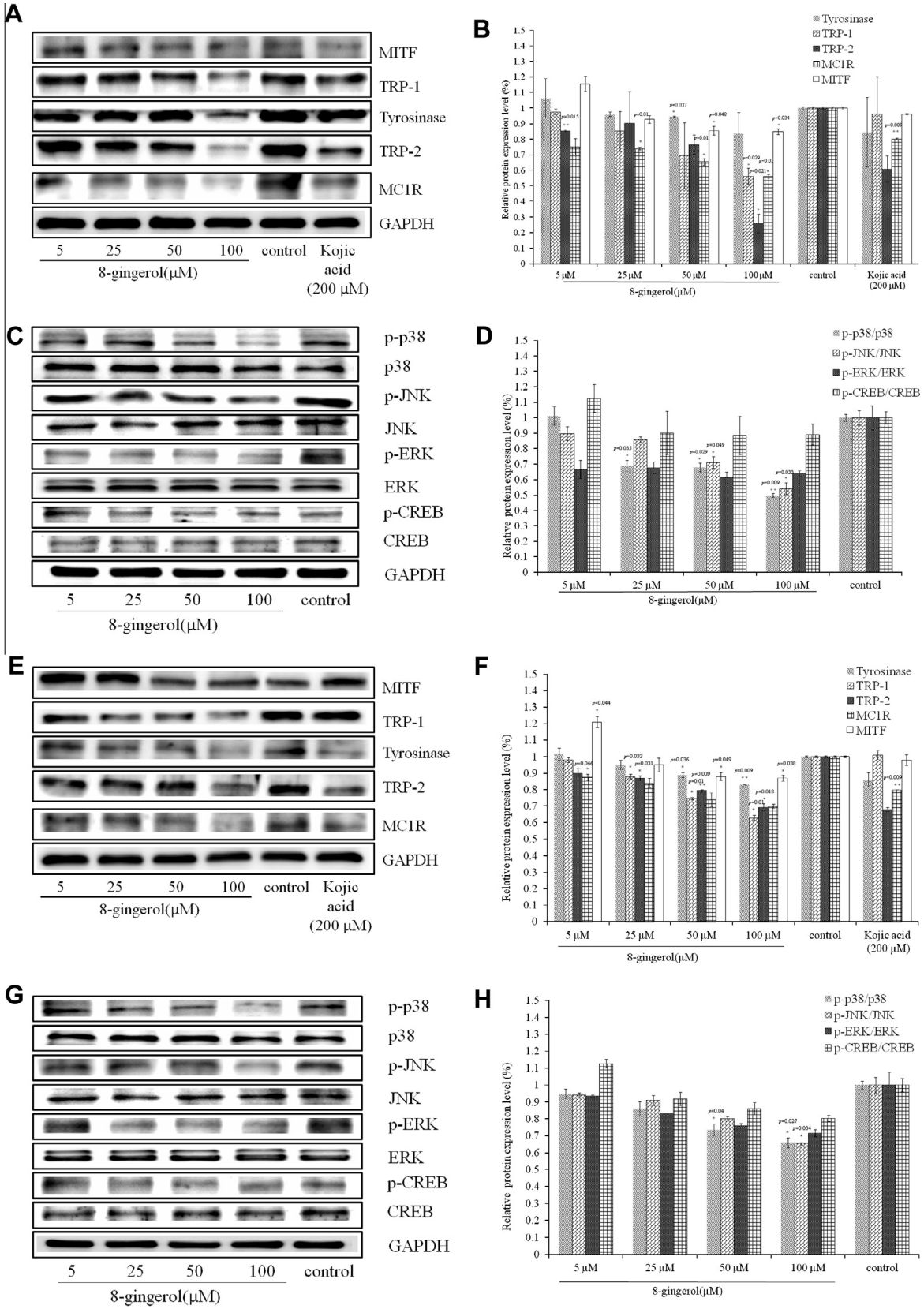

Fig. 2. The effect of [8]-gingerol on melanogenesis-related protein expression and signaling pathways. (A, C, E, G) Western blotting of B16F10 and B16F1 cells. (B, D, F, H) The relative amounts of MITF, TRP-1, tyrosinase, TRP-2 and MC1R compared to the total GAPDH, or phosphorylated protein compared to un-phosphorylated protein were calculated and analyzed using Multi Gauge 3.0 software, and the values represented the mean of triplicate experiments ± standard deviations. $^{*}p < 0.05$; $^{**}p < 0.01$.

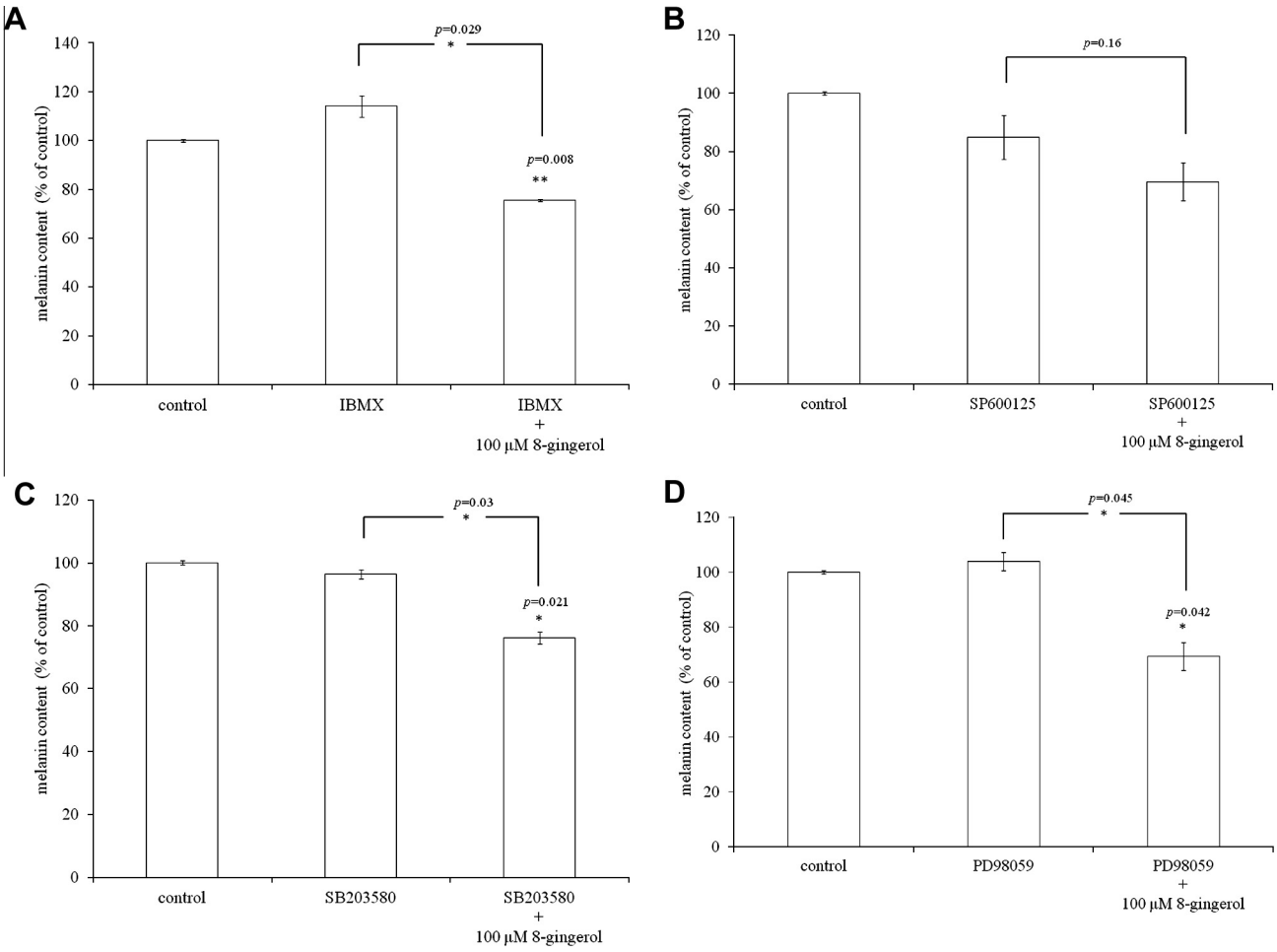

Fig. 3. The effects of [8]-gingerol on melanin content in IBMX, SP600125, SB203580, PD98059-treated B16F10 cells. (A) The melanin content of IBMX-treated cells and (IBMX+[8]-gingerol)-treated cells. (B) The melanin content of SP600125- and (SP600125 + [8]-gingerol)-treated cells. (C) The melanin content of SB203580- and (SB203580 + [8]-gingerol)-treated cells. (D) The melanin content of PD98059-treated cells and (PD98059 + [8]-gingerol)-treated cells. The results are represented as percentages of the control, and the data are presented as the mean ± S.D. of three separate experiments. The values are significantly different compared with the control. $^{*}p < 0.05$; $^{**}p < 0.01$.

3.4. [8]-Gingerol down-regulated JNK-mediated signaling pathway

The JNK signaling pathway is involved in regulating melanogenesis. The results shown in Fig. 2C, reveal that [8]-gingerol decreased the expression of p-JNK; the ratios of p-JNK/JNK in B16F10 cells were 0.89, 0.86, 0.71 and 0.54 for the 5, 25, 50 and 100 μM [8]-gingerol treatments, respectively (Fig. 2D). Besides, the ratios of p-JNK/JNK in B16F1 cells were 0.94, 0.91, 0.8 and 0.65 for the 5, 25, 50 and 100 μM [8]-gingerol treatments, respectively (Fig. 2H). The addition of [8]-gingerol to SP600125-treated B16F10 cells significantly decreased the cellular melanin content. The results indicate that the JNK-mediated signaling pathway, which is involved in melanin production, was affected by [8]-gingerol (Fig. 3B).

3.5. [8]-Gingerol down-regulated p38 MAPK signaling pathway

To further investigate the role of p38 MAPK signaling on the [8]-gingerol-induced anti-melanogenic effect, we employed a specific inhibitor of p38, SB203580, which blocks p38 MAPK signaling. The cells were pre-treated with 10 μM SB203580 in the presence of 100 nM α-MSH and 5–100 μM of [8]-gingerol, and the melanin content was then measured. As shown in Fig. 2C, various concentrations of [8]-gingerol decreased the expression p-p38; the ratios of p-p38/p38 in B16F10 cells were 1.01, 0.69, 0.68 and 0.49 for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively (Fig. 2D). The ratios of p-p38/p38 in B16F1 cells were 0.95, 0.86, 0.74 and 0.66 for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively (Fig. 2H). The results in Fig. 3C, reveal that the specific inhibitor of p38 MAPK, SB203580, attenuated α-MSH-stimulated melanin synthesis. These results suggest that [8]-gingerol inhibited melanin synthesis by down-regulating p38 MAPK signaling and, subsequently, decreased melanin synthesis in α-MSH-stimulated B16F10 cells.

3.6. [8]-Gingerol down-regulated ERK-mediated signaling pathway

The ERK signaling pathway is involved in regulating melanogenesis. The results shown in Fig. 2C, reveal that [8]-gingerol decreased the expression of p-ERK; the ratios of p-ERK/ERK in B16F10 cells were 0.67, 0.66, 0.61 and 0.59 for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively (Fig. 2D). The ratios of p-ERK/ERK in B16F1 cells were 0.93, 0.83, 0.76 and 0.72 for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively (Fig. 2H). The addition of [8]-gingerol in PD98059-treated B16F10 cells significantly decreased the cellular melanin content. The results indicate that the ERK-mediated signaling pathway is involved in melanin production was affected by [8]-gingerol treatment (Fig. 3D).

3.7. [8]-Gingerol depleted the levels of ROS and RS in B16F10 and B16F1 cells

In the ROS assay, [8]-gingerol significantly depleted the intracellular ROS content, and the remaining ROS levels in B16F10 cells were 97.21 ± 0.95%, 91.04 ± 4.36%, 82 ± 3.35% and 71.01 ± 1.45% for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively. The ROS level for the positive standard Trolox® (2 mM) was 66.69 ± 1.68%. Simultaneously, the remaining ROS levels in B16F1 cells were 99.11 ± 1.34%, 93.25 ± 3.27%, 86.53 ± 2.82% and 77.18 ± 2.54% for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively. The ROS level for Trolox® (2 mM) was 70.42 ± 2.23% (Fig. 4A). Additionally, [8]-gingerol also significantly depleted B16F10 intracellular RS levels. The remaining RS levels were 91.04 ± 0.79%, 83.61 ± 3.95%, 77.95 ± 1.87% and 74.96 ± 3.51% for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively. The RS level for Trolox® (0.2 mM) was 68.6 ± 0.8%. The remaining RS levels in B16F1 cells were 95.44 ± 1.48%, 90.67 ± 1.89%, 85.28 ± 2.37% and 80.39 ± 1.92% for the 5, 25, 50 and 100 μM of [8]-gingerol treatments, respectively. The RS level for Trolox® (0.2 mM) was 75.54 ± 1.75% (Fig. 4B). The results shown in Fig. 4 reveal that [8]-gingerol significantly suppressed intracellular ROS and RS production. Therefore, [8]-gingerol was able to protect melanoma cells from oxidative injury by depletion of ROS and RS generation and may down-regulate UV-induced melanogenesis.

4. Discussion

In the cell viability assay, it is found that the tested concentrations of [8]-gingerol (5, 25, 50 and 100 μM) showed no cytotoxicity to the B16F10, B16F1 and HaCaT cells (data not shown). In the present study, α-MSH was used as a cAMP inducer to stimulate melanin synthesis. Evidence has shown that α-MSH can bind MC1R and activate adenylate cyclase, which in turn catalyzes ATP to cAMP and increases intracellular cAMP levels [25]. It has also been reported that binding of the human MC1R by its ligands can activate the cAMP signaling pathway and regulate pigmentation of human melanocytes [26].

In mammals, melanin biosynthesis is directly regulated by three major enzymes, tyrosinase, TRP-1 and TRP-2 [27]. Furthermore, MITF is known to be the most important regulator of melanocyte differentiation and pigmentation [28] and is the major transcriptional regulator of the tyrosinase, TRP-1 and TRP-2 genes. The results shown in Fig. 2 indicate that [8]-gingerol decreased the protein expression levels of those proteins and inhibited tyrosinase activity, in turn decreasing the melanin content in B16F10 cells. Fig. 2 indicates that [8]-gingerol decreased MC1R expression and further suggests that [8]-gingerol inhibited melanogenesis induced via α-MSH-mediated intracellular cAMP up-regulation. Moreover, the results shown in Fig. 3 further confirm that [8]-gingerol inhibited cAMP-mediated PKA signaling.

The MAPK family comprises three types of protein kinases, including extracellular responsive kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK. It has been reported that MAPKs can act to modulate melanogenesis [29–31]. It has been reported that p38 activation positively contributes to melanin production [32] by activating the cAMP response element-binding protein (CREB), and CREB activates MITF expression [33]. The results in Fig. 3 provide evidence that [8]-gingerol could inactivate CREB, JNK and p38, in turn inhibiting MITF expression (Fig. 2). Furthermore, it has been reported that protein kinase A (PKA) signaling is involved in melanin production [34]. The elevation of cellular cAMP levels could activate PKA. In turn, activated PKA can activate CREB, leading to the activation of MITF transcriptional activity and resulting in the expressions of melanogenesis-related proteins. Our results shown in Fig. 3 also suggest that [8]-gingerol inhibits melanin synthesis through blocking of the PKA pathway.

The skin is exposed to UV radiation and environmental oxidizing pollutants and is a preferred target of oxidative stress. It has been reported that ultraviolet irradiation induces the formation of RS and ROS in cutaneous tissue, provoking toxic changes, such as lipid peroxidation and enzyme inactivation [35]. To counteract the oxidative damage, the skin is equipped with a network of enzymatic and non-enzymatic antioxidant systems. The results shown in Fig. 4 suggest that the [8]-gingerol-induced decrease in melanin production may be attributed to its depletion of cellular RS and ROS.

This is the first report regarding the effect of [8]-gingerol on melanin production. In the present study, it was determined that [8]-gingerol significantly inhibits tyrosinase activity and decreases melanin synthesis. Moreover, [8]-gingerol also expresses intracellular free radical scavenging activity. The results suggest that [8]-gingerol decreases melanin production, which may be attributed to its inhibitory action upon the signaling pathway that regulates tyrosinase activity or by the depletion of cellular RS and ROS. Further, our results demonstrate that [8]-gingerol decreases melanogenesis in melanoma cells by inactivating PKA and MAPK signaling pathways, reducing MITF expression and inhibiting tyrosinase activity. Additionally, the inhibitory effect of [8]-gingerol on melanin production may also be mediated by the depletion of the intracellular ROS and RS contents.

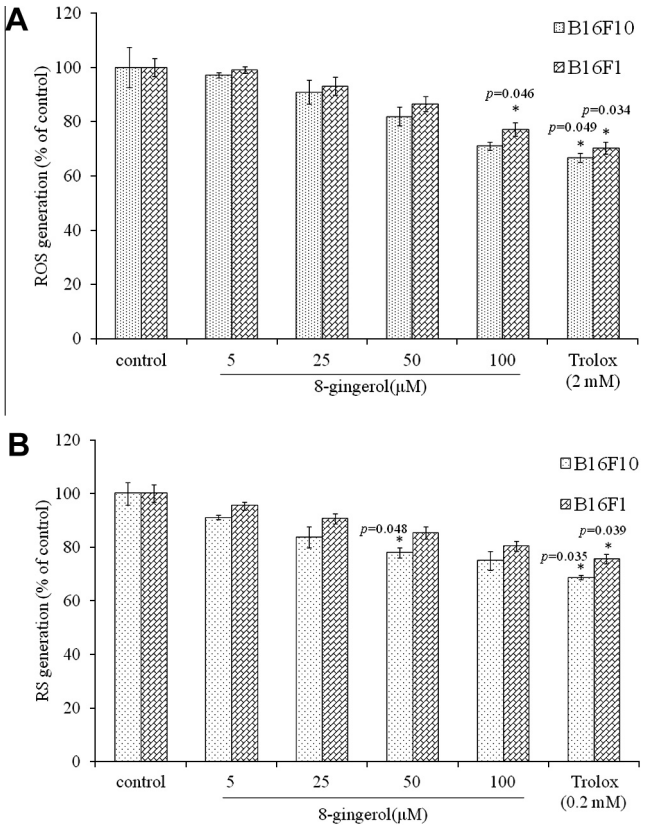

Fig. 4. The effect of [8]-gingerol on the RS and ROS content in B16F10 and B16F1 cells. (A) [8]-Gingerol decreased the cellular ROS level. (B) [8]-Gingerol depleted the cellular RS content. The results are expressed as percentages of the control. The data are presented as the mean ± S.D. $^{*}p < 0.05$.

Acknowledgment

This study was financially supported by the National Science Council, Taiwan, under Grant Nos. NSC 101-2918-I-039-004 and NSC 101-2221-E-241-013.

References

[1] S. Briganti, E. Camera, M. Picardo, Chemical and instrumental approaches to treat hyperpigmentation, Pigment Cell Res. 16 (2003) 101–110.
[2] S.-Y. Seo, V.K. Sharma, N. Sharma, Mushroom tyrosinase: recent prospects, J. Agric. Food Chem. 51 (2003) 2837–2853.
[3] V.J. Hearing, M. Jiménez, Mammalian tyrosinase – the critical regulatory control point in melanocyte pigmentation, Int. J. Biochem. 19 (1987) 1141–1147.
[4] C. Jiménez-Cervantes, F. Solano, T. Kobayashi, et al., A new enzymatic function in the melanogenic pathway. The 5,6-dihydroxyindole-2-carboxylic acid oxidase activity of tyrosinase-related protein-1 (TRP1), J. Biol. Chem. 269 (1994) 17993–18000.
[5] K. Tsukamoto, I.J. Jackson, K. Urabe, et al., A second tyrosinase-related protein, TRP-2, is a melanogenic enzyme termed DOPAchrome tautomerase, EMBO J. 11 (1992) 519–526.
[6] J.C. García-Borrón, B.L. Sánchez-Laorden, C. Jiménez-Cervantes, Melanocortin-1 receptor structure and functional regulation, Pigment Cell Res. 18 (2005) 393–410.
[7] Y. Funasaka, M. Komoto, M. Ichihashi, Depigmenting effect of α-Tocopheryl ferulate on normal human melanocytes, Pigment Cell Res. 13 (2000) 170–174.
[8] J. Yamakoshi, F. Otsuka, A. Sano, et al., Lightening effect on ultraviolet-induced pigmentation of Guinea pig skin by oral administration of a proanthocyanidin-rich extract from grape seeds, Pigment Cell Res. 16 (2003) 629–638.
[9] G. Imokawa, Analysis of initial melanogenesis including tyrosinase transfer and melanosome differentiation though interrupted melanization by glutathione, J. Invest. Dermatol. 93 (1989) 100–107.
[10] Y. Kumano, T. Sakamoto, M. Egawa, et al., In vitro and in vivo prolonged biological activities of novel vitamin C derivative, 2-O-alpha-D-glucopyranosyl-L-ascorbic acid (AA-2G), in cosmetic fields, J. Nutr. Sci. Vitaminol. (Tokyo) 44 (1998) 345–359.
[11] Y.J. Kim, J.K. No, J.H. Lee, et al., 4,4′-Dihydroxybiphenyl as a new potent tyrosinase inhibitor, Biol. Pharm. Bull. 28 (2005) 323–327.
[12] C. Manach, A. Scalbert, C. Morand, et al., Polyphenols: food sources and bioavailability, Am. J. Clin. Nutr. 79 (2004) 727–747.
[13] O. Nerya, J. Vaya, R. Musa, et al., Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots, J. Agric. Food Chem. 51 (2003) 1201–1207.
[14] H.-C. Huang, S.-H. Chiu, T.-M. Chang, Inhibitory effect of [6]-gingerol on melanogenesis in B16F10 melanoma cells and a possible mechanism of action, Biosci. Biotechnol. Biochem. 75 (2011) 1067–1072.
[15] J. Lu, S. Guan, X. Shen, et al., Immunosuppressive activity of [8]-gingerol on immune responses in mice, Molecules 16 (2011) 2636–2645.
[16] M. Kobayashi, Y. Ishida, N. Shoji, et al., Cardiotonic action of [8]-gingerol, an activator of the Ca++-pumping adenosine triphosphatase of sarcoplasmic reticulum, in Guinea pig atrial muscle, J. Pharmacol. Exp. Ther. 246 (1988) 667–673.
[17] S. Dugasani, M.R. Pichika, V.D. Nadarajah, et al., Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol, J. Ethnopharmacol. 127 (2010) 515–520.
[18] P. Boukamp, T. Petrussevska, D. Breitkreutz, et al., Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line, J. Cell Biol. 106 (1988) 761–771.
[19] H. Tada, O. Shiho, K. Kuroshima, et al., An improved colorimetric assay for interleukin 2, J. Immunol. Methods 93 (1986) 157–165.
[20] M.L. Bilodeau, J.D. Greulich, R.L. Hullinger, et al., BMP-2 stimulates tyrosinase gene expression and melanogenesis in differentiated melanocytes, Pigment Cell Res. 14 (2001) 328–336.
[21] T. Tsuboi, H. Kondoh, J. Hiratsuka, et al., Enhanced melanogenesis induced by tyrosinase gene-transfer increases boron-uptake and killing effect of boron neutron capture therapy for amelanotic melanoma, Pigment Cell Res. 11 (1998) 275–282.
[22] J.Y. Yang, J.H. Koo, Y.G. Song, et al., Stimulation of melanogenesis by scoparone in B16 melanoma cells, Acta Pharmacol. Sin. 27 (2006) 1467–1473.
[23] C.L. Murrant, M.B. Reid, Detection of reactive oxygen and reactive nitrogen species in skeletal muscle, Microsc. Res. Tech. 55 (2001) 236–248.
[24] T. Paraidathathu, H. de Groot, J.P. Kehrer, Production of reactive oxygen by mitochondria from normoxic and hypoxic rat heart tissue, Free Radic. Biol. Med. 13 (1992) 289–297.
[25] R. Buscà, R. Ballotti, Cyclic AMP a key messenger in the regulation of skin pigmentation, Pigment Cell Res. 13 (2000) 60–69.
[26] Z. Abdel-Malek, V. Swope, C. Collins, et al., Contribution of melanogenic proteins to the heterogeneous pigmentation of human melanocytes, J. Cell Sci. 106 (1993) 1323–1331.
[27] K. Kameyama, C. Sakai, S. Kuge, et al., The expression of tyrosinase, tyrosinase-related proteins 1 and 2 (TRP1 and TRP2), the silver protein, and a melanogenic inhibitor in human melanoma cells of differing melanogenic activities, Pigment Cell Res. 8 (1995) 97–104.
[28] C. Levy, M. Khaled, D.E. Fisher, MITF: master regulator of melanocyte development and melanoma oncogene, Trends Mol. Med. 12 (2006) 406–414.
[29] N. Hirata, S. Naruto, K. Ohguchi, et al., Mechanism of the melanogenesis stimulation activity of (−)-cubebin in murine B16 melanoma cells, Bioorg. Med. Chem. 15 (2007) 4897–4902.
[30] D.S. Kim, Y.M. Jeong, I.K. Park, et al., A new 2-imino-1,3-thiazoline derivative, KHG22394, inhibits melanin synthesis in mouse B16 melanoma cells, Biol. Pharm. Bull. 30 (2007) 180–183.
[31] K. Smalley, T. Eisen, The involvement of p38 mitogen-activated protein kinase in the α-melanocyte stimulating hormone (α-MSH)-induced melanogenic and anti-proliferative effects in B16 murine melanoma cells, FEBS Lett. 476 (2000) 198–202.
[32] S.K. Singh, C. Sarkar, S. Mallick, et al., Human placental lipid induces melanogenesis through p38 MAPK in B16F10 mouse melanoma, Pigment Cell Res. 18 (2005) 113–121.
[33] M.-D. Galibert, S. Carreira, C.R. Goding, The Usf-1 transcription factor is a novel target for the stress-responsive p38 kinase and mediates UV-induced tyrosinase expression, EMBO J. 20 (2001) 5022–5031.
[34] T. Hirobe, Basic fibroblast growth factor stimulates the sustained proliferation of mouse epidermal melanoblasts in a serum-free medium in the presence of dibutyryl cyclic AMP and keratinocytes, Development 114 (1992) 435–445.
[35] I. Emerit, Free radicals and aging of the skin, EXS 62 (1992) 328–341.