Thesis for the Degree of Science

Bieckol isolated from *Ecklonia stolonifera* inhibits melanin synthesis in B16 melanoma cells

By

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Pukyong National University

February 2010

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*E. stolonifera*에서 분리된 bieckol의 B16 melanoma cell에서의 멜라닌 합성 억제효과

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By

Min Do Kim

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A dissertation

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Contents

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Abstract

Melanin has major roles in skin and pigmented epithelium of the retina. Melanin plays a role in protection against DNA damages and photo-carcinogenesis caused by exposure of the solar-radiation in human skin. Although, melanin is excellent photo-protectant to prevent the DNA damages by UV-radiation, mis-regulation of production and accumulation of melanin is leads to hyper-pigmentation in skin. Melanin is synthesized in the melanosomes of melanocytes and, regulated by melanogeninc enzymes, such as tyrosinase, tyrosinase related protein-1 (TRP-1), and tyrosinase related protein-2 (TRP-2). In this study, the inhibitory effects of the biekol isolated from *E. stolonifera* on α-MSH-stimulated melanogenesis in

cultured B16 mouse melanoma cells were investigated. Biekol had no inhibitory cytotoxicity on B16 cells up to 10 μM and intracellular tyrosinase activity was suppressed by bieckol treatment with dose-dependent manner. In Western blotting analysis, biekol inhibited the expression of tyrosinase, TRP-1, and TRP-2 in α-MSH-stimulated cells with a dose-dependent manner. Furthermore, in RT-PCR experiments, the mRNA expressions of TRP-1, TRP-2 and MITF were significantly reduced by biekol treatment with dose-dependent manner. Bieckol treatment with 5 μM delayed the phosphorylation of Akt and ERK which are responsible to the degradation of MITF. In this result, the hypopigmentary effect of biekol isolated from *E. stolonifera* resulted from the inhibition of the expression of tyrosinase, TRP-1, and TRP-2 via down-regulation of MITF through phosphorylation of Akt and ERK. Bieckol may provide a beneficial effect for inhibiting melanogenesis and it has a potential for the development of functional cosmetics for whitening effects.

*Ecklonia stolonifera*에서 분리된 bieckol의 B16 melanoma cell에서의 멜라닌 합성 억제효과

국문 초록

멜라닌은 인체 피부와 모발의 주요 색깔의 결정에 관여 하는 색소 성분으로서 이러한 멜라닌의 합성과정은 멜라닌세포에 존재하는 멜라노좀에서 합성되며, 이러한 과정은 타이로시네이즈나 타이로시네이즈 관련 단백질 1, 2 등에 의해 조절 된다. 본 연구에서는 해조류인 *Ecklonia stolonifera* 에서 분리된 biekol 을 배양된 마우스 흑색 종 세포주인 B16 세포에서 α-MSH 자극으로 유발된 멜라닌합성에 대한 영향을 알아 보고자 하며 또한, 그 작용기전에 대하여 알아보고자 한다. MTS 실험결과 10 μM 의 농도 내에서 biekol 은 흑색 종 세포의 증식에 영향을 주지 않았다. B16 세포에 bieckol 을 처리한 결과 5 μM 까지 농도의존적으로 멜라닌

합성이 억제되었으며, 더불어 tyrosinase 의 활성도 농도의존적으로 저해되었다. Western blotting 에서 biekol 은 tyrosinase, TRP-1 과, TRP-2 의 발현을 농도에 비례하여 억제하였으며, 이러한 현상이 RT-PCR 분석결과 mRNA 에서도 동일한 결과로 농도에 비례하여 나타났다. *Ecklonia stolonifera* 에서 분리된 biekol 은 α-MSH 로 자극한 B16 흑색 종 세포의 멜라닌 합성 과정에서 tyrosinase, TRP-1 과 TRP-2 의 발현을 농도에 비례하여 유의하게 감소시켰으며, mRNA 전사수준에서도 동일하게 나타났다. 또한 MITF 의 인산화에 관여하였으며, Akt 와 ERK 의 인산화를 bieckol 이 지연시키는 것으로 나타났다. 이상의 결과로 추정컨데, α-MSH 로 자극된 B16 흑색종 세포에 bieckol 을 처리함으로써 Akt 와 ERK 의 인산화로 인한 MITF 의 분해와 타이로시네이즈 및 타이로시네이즈 관련 단백질 1 과 2 를 전사 수준에서 발현을 억제하여 피부 미백 효과를 나타내는 것으로 판단 된다.

Introduction

Melanin is ubiquitous pigments that, in mammals, are found in many different organs of the body. In humans, melanin has major roles in the skin where they are responsible for color in the eyes where it is present in the iris, and in the pigmented epithelium of the retina [1]. Melanin plays a role in protection against DNA damage and photo-carcinogenesis caused by severe exposure to solar radiation in human skin. Although melanin is an excellent photo-protectant to prevent the DNA damage by absorbing excessive UV-radiation, mis-regulation of production and accumulation of melanin leads to hyper-pigmentation in skin [2]. Therefore, interests for whitening effects of cosmetics which can control pigmentation have been increased and many studies have been reported that natural materials extracted from plants have whitening effects by inhibiting melanogenesis in skin. In recent studies, several enzymes that participate in melanin synthesis have been clarified and inhibition of these enzymes is targeted to study for whitening function of natural Compounds.

For the melanin synthesis, there are three well-known enzymes, tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2). Tyrosinase has an important role in melanogenesis

that catalyzes three different reactions: the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), the oxidation of DOPA to DOPA quinone, and the oxidation of 5,6-dihydroxyindole (DHI) to indole-quinone. In the absence of thiols, DOPA quinnone changes to DOPA chrome and then to DHI or indole 5, 6-quinone-2-carboxylic acid (DHICA). TRP-2 isomerizes DOPA chrome to DHICA. TRP-1 has been shown in mice to produce DHICA to indole 5, 6-quinone-2-carboxylic acid, while human TRP-1 seems to be devoid of this activity [3, 4].

Several signal pathways are participated in enhancing melanin production. In melanocytes, nitric oxide (NO) induced by UVB irradiation activates tyrosinase to stimulate melanogenesis through gauntly cyclase, cyclic GMP (cGMP), and protein kinase C (PKC) [4]. α-Melanocyte stimulating hormone (α-MSH) activates adenylyl cyclase through G proteins to increase intracellular cyclic AMP (cAMP) content. cAMP induces a complex set of intracellular processes that activates PKA and cAMP responsive element binding protein (CREB) to up-regulate MITF expression [4]. MITF strongly stimulates tyrosinase and TRP-1 promoter activities, indicating that MITF is an important transcriptional regulator in melanogenesis [5].

Brown algae are very popular as seafood and many people ingest them as a health food in Korea and Japan. *Ecklonia stolonifera*

OKAMURA, the representative brown algae of *Ecklonia sp.*, is a member of the family of Laminariaceae. Phlorotannins, the secondary metabolites of brown algae, are known to have several biological activities such as anti-oxidation [6], anti-mutation [7], anti-skin aging [8], and mushroom tyrosinase inhibitory activity [9]. Among the phlorotannins isolated from *Ecklonia stolonifera* OKAMURA, bieckol have been reported to have biological activities including anti-oxidation, anti-plasmin, anti-inflammation, bactericide, HIV-1 reverse-transcriptase and protease inhibition, and photo-chemoprevention [10-17]. A recent study has been reported that 7-phloroeckol serves as a non-competitive inhibitor to mushroom tyrosinase and melanin synthesis was inhibited in melanoma cells [18]. However the effect of the compound on protein and mRNA expression level was not cleanified.

In this study, the effects of bieckol on the suppression of melanogenesis were investigated in the gene transcriptional and protein translational levels using α-MSH-stimulated B16 F10 melanoma cells.

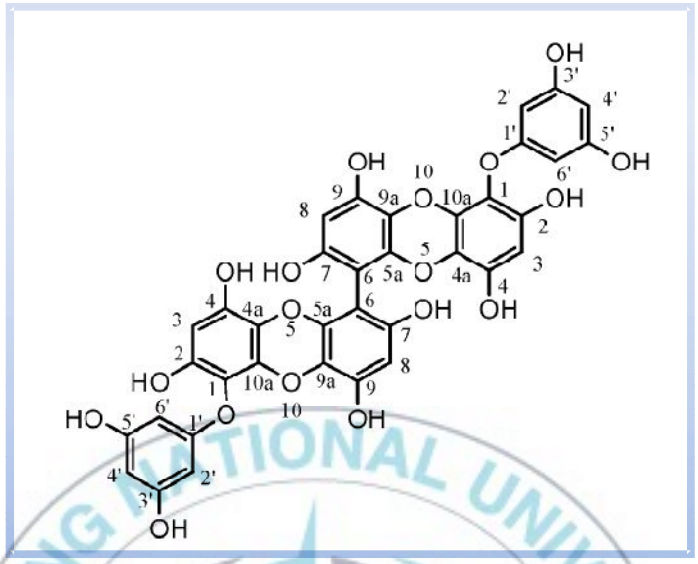

Fig. 1. The structure of bieckol

2. Materials and Methods

2.1. Materials

DMEM (Dulbecco's Modified Eagle's Medium) was purchased from WelGENE Inc. (Daegu, South Korea). Penicillin-streptomycin mixture, 0.25% tripsin-EDTA, fetal bovine serum (FBS) was purchased from HyClone Laboratory Inc. (Logan, UT), CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay was purchased from Promega (Madison, WI). α-MSH, $_{L}$-DOPA and DMSO (dimethyl sulfoxide) were purchased from Sigma Chemical (St. Louis, MO). TRIzol reagent was purchased from Invitrogen (Grand Island, NY). ECL detection kit was purchased from AbFrontier (Seoul, South Korea). Polyclonal antibodies against tyrosinase, TRP-1, TRP-2, MITF, ERK1/2, phosphor-ERK1/2 (pERK1/2), Akt, phosphor-Akt and β-actin, and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Bieckol was isolated from *Ecklonia stolonifera* described in Kim et al. [13].

2.2. Tyrosinase activity assay

Tyrosinase activity was determined as previously described [19]. Briefly, B16F10 melanoma cells were cultured in 60 mm dishes. After incubating with different concentration of bieckol in DMEM containing 2% FBS for 3 days, the cells were washed with ice-cold PBS and lysed with phosphate buffer (pH 6.8) containing 1% Triton X-100. The cells were then disrupted by freezing and thawing, and the lysates were clarified by centrifugation at 10,000 x g for 5 min. After quantifying protein levels and adjusting protein concentrations with lysis buffer, 90 μl of each lysate, containing the same amount of protein, was placed in each well of a 96-well plate, and 10 μl of 10 mM $_{L}$-DOPA was added to each well. Control wells contained 90 μl of lysis buffer and 10 μl of 10 mM $_{L}$-DOPA. The absorbance was measured every 10 min for at least 1 hr during incubated at 37℃ at 475 nm using an ELISA reader.

2.3. Cell culture and treatment

B16F10 melanoma cells (ATCC, MD) were maintained in DMEM containing 2.0 mM L-glutamine, Earle's BSS, and 10% heat-inactivated

FBS at 37℃ in 5% CO_2. For induction of melanin synthesis, cells were cultured with 10 nM of α-MSH containing 2% FBS for 3 days. Bieckol was co-treated with α-MSH for identifying effects of bieckol on the suppression of melanin synthesis in B16F10 cells. The final concentration of DMSO did not exceed 0.1%.

2.4. Cell viability assay

Cell viability was determined by MTS assay using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay Kit according to the manufacturer's manual. Pre-confluent B16 melanoma cells were cultured in 96-well plates at a density of 2.5 x 10^4 cells/well in DMEM with 10% FBS. All cells were treated with various concentrations of samples in 2% FBS for 3 days at 37℃ under 5% CO_2. The culture medium was removed and replaced by 95 μl of fresh culture medium and 5 μl of MTS solution. After 1 hr, the absorbance was measured using a microplate reader Ultraspec® 2100 *pro*, Amersham Biosciences, Piscataway, NJ) at 490 nm.

2.5. Measurement of melanin contents and microscopic observation

Melanin contents were measured as described previously by Tsuboi et al.[20] with slight modification. Briefly, cells were treated with test substances in DMEM containing 2% FBS for 3 days. Cell pellets were dissolved in 1 ml of 2 N NaOH at 100℃ for 30 min and centrifuged for 20 min at 16,000 x g. Absorbance of the supernatants was measured at 405 nm using a micro-plates reader. Before measuring the melanin content, the cells were observed under a phase contrast microscope (Olympus Optical Co., Tokyo, Japan) and photographed with an Olympus digital video camera system.

2.6. Western immunoblot analysis

B16F10 cells cultured in 6-well plates for 3 days were washed twice with ice-cold PBS containing protease and phosphatase inhibitors. A lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% SDS, 1% Nonidet P-40, 1 mM EDTA, 1 mM DTT, 200 nM aprotinin, 20 μM leupetin, 50 μM phenanthroline, 280 μM benzamidine

- HCl) was added in each well and stood on ice for 30 min for lysis. After centrifugation of the lysed cells at 12,000 x g for 20 min at 4°C, the protein content of the supernatant was measured, and aliquots (20 μg) of proteins were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% (w/v) non-fat milk powder in TBST (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 1 hr and incubated for 16 hr with the primary antibody in TBST. The blots were treated with the secondary antibody in TBST for 2 hr, and an immune complex was detected using ECL detection kit.

2.7. Reverse transcription-polymerase chain reaction (RT-PCR)

To investigate the effect of bieckol on melanogenic gene expression, RT-PCR analysis was performed. B16F10 melanoma cells were treated with or without bieckol (1 or 5 μM) for 3 days. After treatment, total RNA was extracted from the cells using TRIzol and was reverse-transcribed. Reverse transcription was carried out with 1 μg of isolated total RNA using the RT-PCR kit (ELPIS Biotech, Inc, Daecheon, Korea). All procedures were conducted following the manufacturer's instruction.

After reverse transcription, PCR was performed using 25 amplification cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 7 min. The oligonucleotide primers used for the PCR were as follows:

Tyrosinase forward 5'- AAGGAGAAAATGTTCTTGGC-3';

Tyrosinase reverse 5'-CAAACTTACAGTTTCCGCAG-3';

TRP-1 forward 5'- CAGGAGCCTTCTTTCTCCCT-3';

TRP-1 reverse 5'- ATACACGGACCTCCAAGCAC-3';

TRP-2 forward 5'- AACAACCCTTCCACAGATGC-3';

TRP-1 reverse 5'- CAGGTAGGAGCATGCTAGGC-3';

MITF forward 5'- AAGTCCTTAAGGTGCAGACC-3';

MITF reverse 5'- AGAGTGCGTGTTCATACCTG-3',

GAPDH forward 5'-ACCACAGTCCATGCCATCAC-3';

GAPDH reverse 5'-TCCACCACCCTGTTGCTGTA-3'

2.8. Statistical analysis

Data were expressed as mean ± SD from triplicates of at least three independent experiments. All analyses were done with SPSS (Statistical analysis software package, USA). Statistical significance was established at *P values of* <0.05, and <0.01.

3. Results

3.1. Effect of bieckol on intracellular tyrosinase activity and melanin synthesis in B16 cells

To examine the effect of bieckol on cell proliferation, B16 melanoma cells were incubated with bieckol (0-10 μM) for 3 days. At these concentrations, bieckol had no significant effect on cell proliferation in the presence of 10 nM of α-MSH (Fig. 2).

To measure the intracellular tyrosinase inhibitory activity of bieckol, various concentration of bieckol (0-10 μM) was tested for their inhibitory effect on intracellular tyrosinase activity using $_{L}$-DOPA as a substrate. Bieckol inhibited tyrosinase activity dramatically at 2.5 and 5 μM (72.1% and 51.0%, respectively); however, further inhibitory activity did not appear at 10 μM of bieckol treatment (47.6%). The EC_{50} value was estimated 7.9 μM of bieckol (Fig. 3).

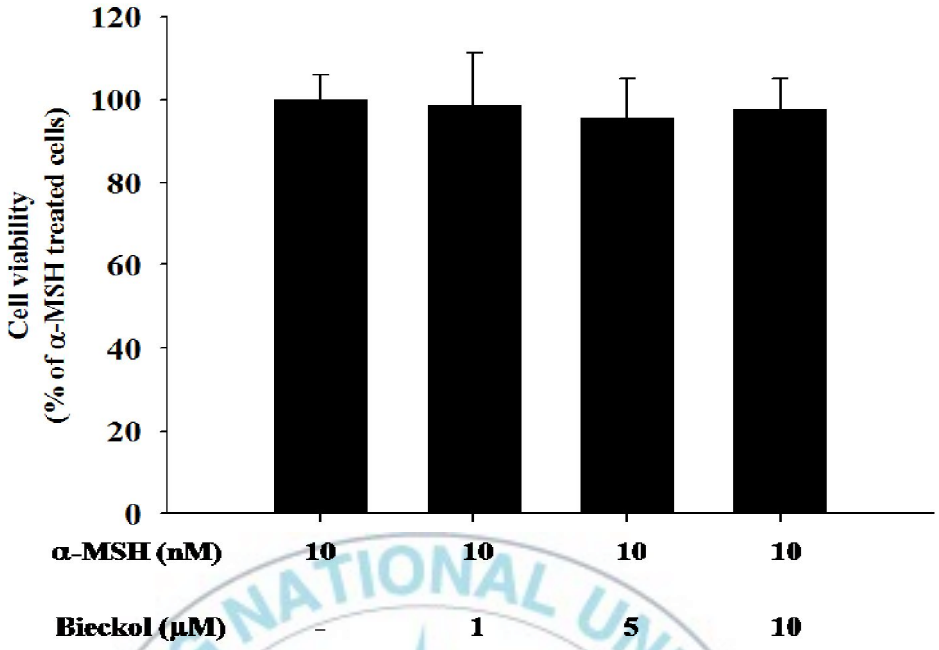

Fig. 2. The effect of bieckol on the proliferation of B16 melanoma cells.

B16 melanoma cells were cultured for 3 days without on with various concentration of bieckol analyzed by MTS assay as described in Materials and Method section. Results are expressed as a percentage of the control culture (α-MSH only), and data are the means ± S.D. of at least three separate experiments.

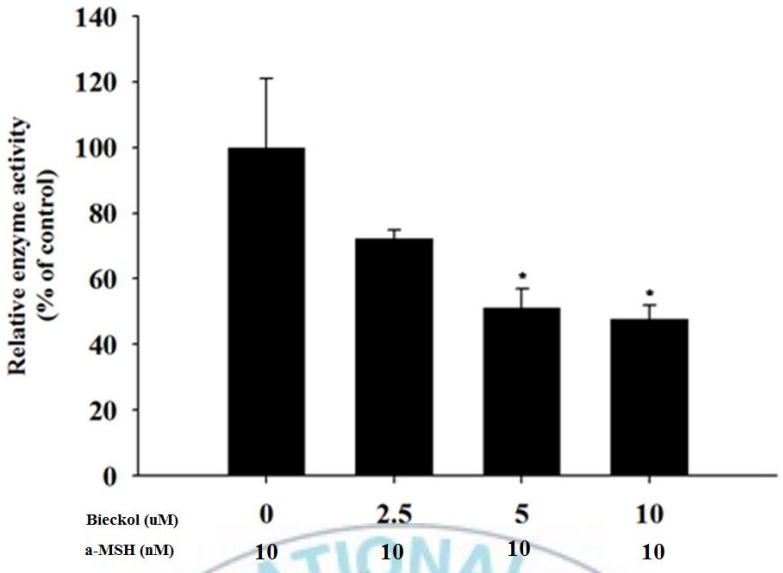

Fig. 3. The effect of bieckol on tyrosinase activity.

Tyrosinase activity was measured as described in Materials and Method section. Results are expressed as a percentage of the control group, and data are the means ± S.D. of at least three separate experiments. *$P < 0.05$ versus control group.

The melanin content of the B16 melanoma cells was photographed under a phase contrast microscope to the determine effects of bieckol on melanin accumulation. The color of the representative cells became whiter as the concentration of bieckol increased (Fig. 4). The melanin content of the B16 cells that had not been treated with α-MSH was low (Fig. 5). Treatment with 1 and 5 μM of bieckol produced an inhibition of melanin production in α-MSH-stimulated B16 melanoma cells (86.8% at 1 μM and 75.2% at 5 μM). However, a progressive inhibitory effect on melanogenesis did not appear at 10 μM of bieckol treatment in B16 melanoma cells (76.9% at 10 μM).

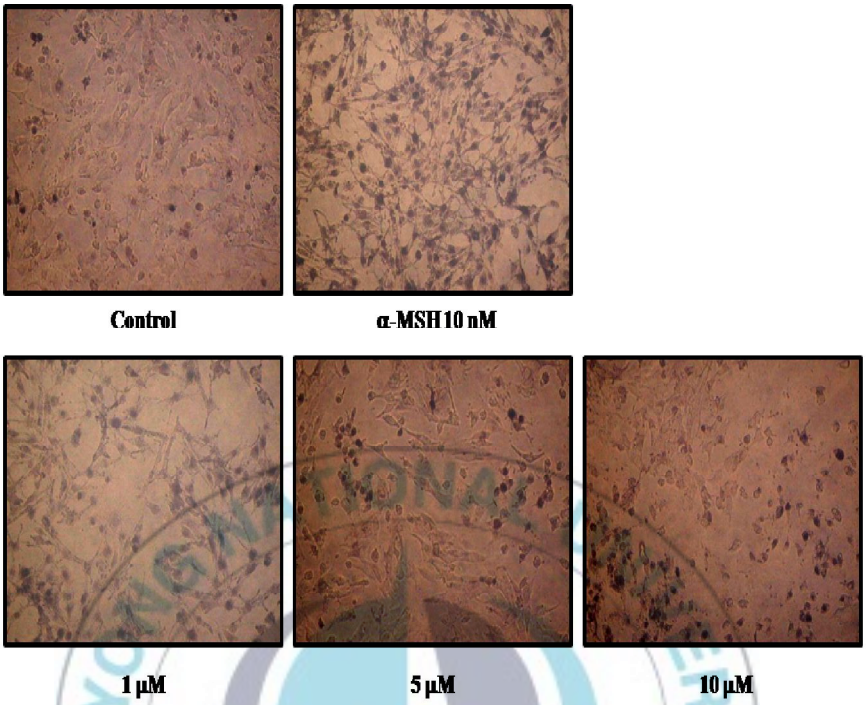

Fig. 4. The effect of bieckol on melanin production.

Photograph of B16 melanoma cells. B16 melanoma cells were cultured for 3 days with or without bieckol, as described in Materials and Method section.

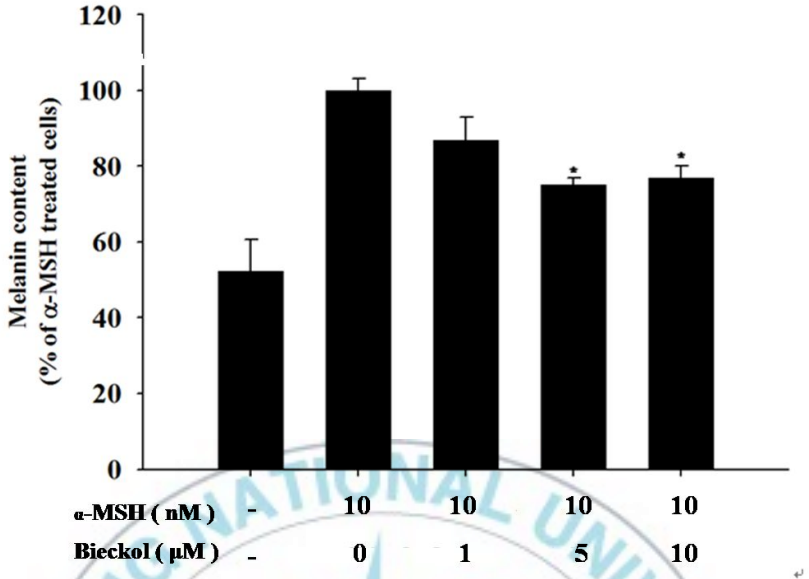

Fig. 5. The effect of bieckol on melanin production.

Results are expressed as a percentage of the control (α-MSH), and data reported are the means ± S.D. of at least three separate experiments. *P < 0.05 versus α-MSH treated group.

3.2. Western blotting of melanogenic proteins

To investigate whether bieckol can influence melanogenic protein expression, Western blotting analysis was carried out using the lysate of α-MSH-stimulated B16 melanoma cells treated with bieckol (Fig. 6). When cells were stimulated by α-MSH alone, a significant increase of tyrosinase, TRP-1, and TRP-2 was observed. Bieckol suppressed the expression of tyrosinase, TRP-1, and TRP-2 in α-MSH-stimulated B16 melanoma cells in a dose-dependent manner. These results suggest that translational changes of proteins are involved in the inhibitory effect of bieckol in the α-MSH-stimulated expression of tyrosinase, TRP-1, and TRP-2. Although the expression of MITF was not stimulated by α-MSH, the protein decreased dose-dependently by bieckol treatment.

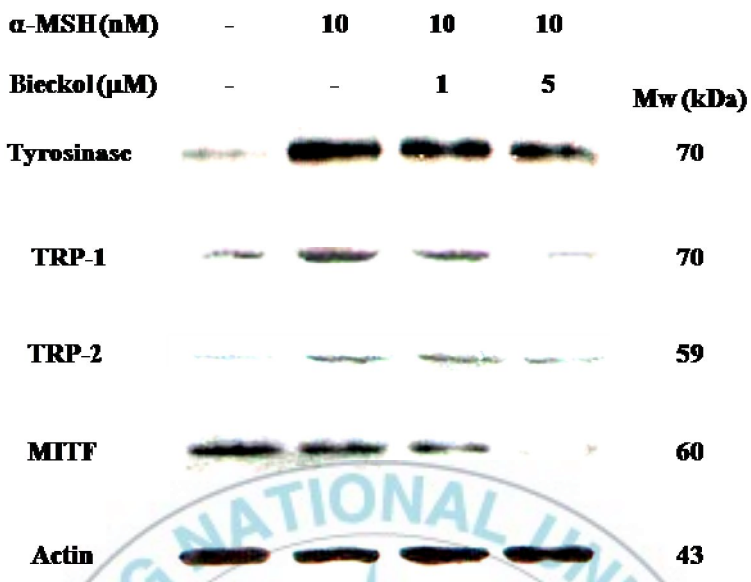

Fig. 6. The effect of bieckol on melanogenic protein expression.

B16 melanoma cells were treated with or without bieckol and stimulated with α-MSH (10 nM). After treatment, Western blotting of tyrosinase, TRP-1, TRP-2, and MITF was performed as described in Materials and Method section. The loading control was assessed using anti-β-actin antibody.

3.3. Bieckol down-regulated melanogenic gene transcription

Regulation of the protein levels could reflect by altered protein synthesis and degradation. RT-PCR experiments were carried out to confirm whether the down-regulation of proteins related to melanogenesis by bieckol was due to a decreased in their mRNA expression. As shown in (Fig. 7), mRNA levels of tyrosinase and TRP-1 decreased in a dose-dependent manner. However, mRNA expressions of TRP-2 and MITF were significantly reduced by treatment of 5 μM bieckol. The gene expression level of GAPDH used as an internal control showed no change.

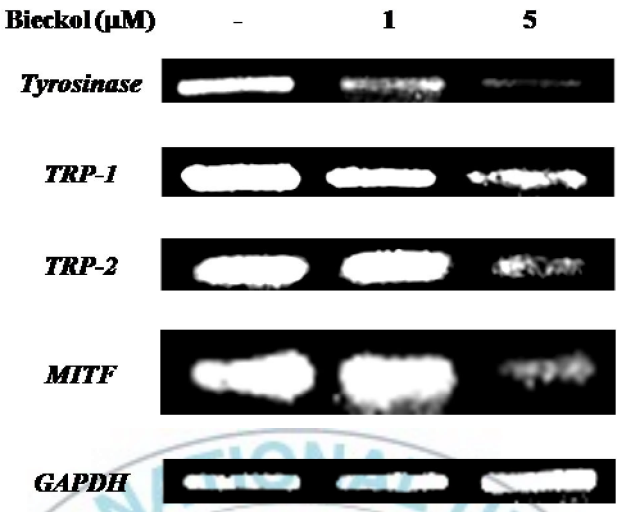

Fig. 7. RT-PCR analysis of melanogenic mRNA levels in bieckol treated B16 melanoma cells.

Cells were treated with bieckol (1 or 5 μM) for 3 days. Total RNA was extracted and cDNA was prepared. Equivalent amounts of cDNA were amplified using primers specific for tyrosinase, TRP-1, TRP-2, MITF, and GAPDH. The level of GAPDH mRNA was checked as a control to ensure the even loading of target cDNA.

3.4. Bieckol induces the phosphorylation of ERK

To clarify whether bieckol could influence the ERK- and the Akt-pathways, we stimulated B16 melanoma cells by 10 nM of α-MSH with 5 μM of bieckol for 0-9 hr. As shown in (Fig. 8), ERK1 and ERK2 were phosphorylated by bieckol in a time-dependent manner, and strongly activated at 6 and 9 hr. Although the activation level was decreased at 3 and 6 hr, Akt also was phosphorylated by bieckol, and strongly activated at 9 hr. In addition, we checked tyrosinase protein expression for the same period, and we found that the tyrosinase protein expression level decreased at 6 and 9 hr.

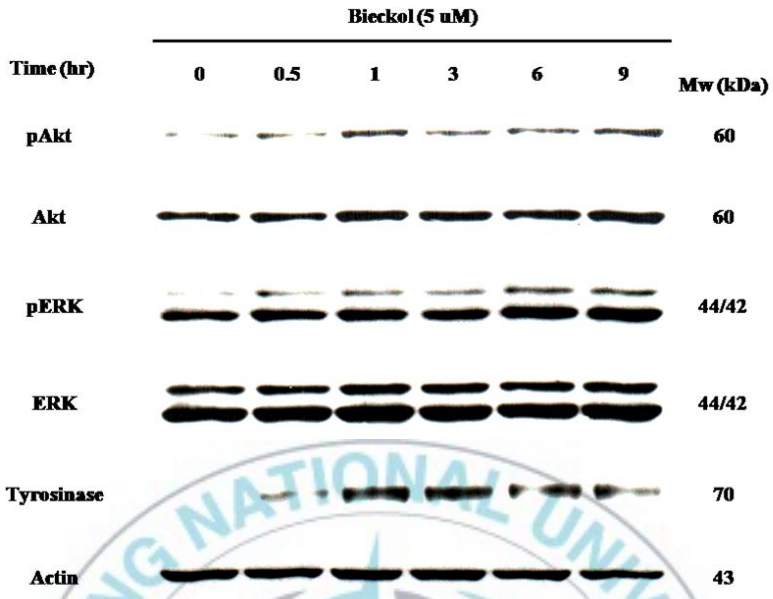

Fig. 8. The effect of bieckol on ERK1/2 and Akt phosphorylation.

B16 melanoma cells were treated with or without bieckol and stimulated with α-MSH (10 nM). After treatment, Western blotting was performed as described in Materials and method section. The loading control was assessed using anti-β-actin, anti-ERK1/2 and anti-Akt antibody.

4. Discussion

In this study, we have firstly demonstrated the inhibitory mechanism of bieckol isolated from *E. stolonifera* in α-MSH-stimulated B16F10 melanoma cells. Bieckol did not have any effect on the cell proliferation up to 10 μM. However, bieckol did inhibit melanin production in α-MSH-stimulated B16F10 melanoma cells. Many in vitro studies showed that several phytochemicals including arbutin, kaemperol, EGC, and EGCG affect mushroom or mammalian tyrosinase activity, which suggest to a potential for a skin whitening agent [21-26]. Several phlorotannins were isolated from *Ecklonia stolonifera* and reported to have an inhibitory activity of mushroom tyrosinase [9], 7-phloroeckol isolated from *Ecklonia cava* inhibits mushroom tyrosinase activity and decreases melanin content in 3-isobutyl-1-methylxanthine (IBMX)-induced B16F10 melanoma cells [19].

There are three well-known enzymes, such as tyrosinase, TRP-1, and TRP-2, related to the procedure of melanin synthesis, and one transcriptional factor, MITF, regulating production of the melanogenic enzymes [3-5]. Tyrosinase inhibitory assay using $_{L}$-DOPA as substrate was performed to confirm to inhibitory activity of bieckol on mammalian tyrosinase activity. In the tyrosinase activity assay using

intracellular system, bieckol inhibited cellular tyrosinase activity. Therefore, this result suggested that bieckol might be suppressed tyrosinase expression or inhibited tyrosinase activity. To confirm tyrosinase inhibitory activity was resulted from direct enzyme inhibition or reduced expression of tyrosinase, Western blot analysis was performed the expression of proteins associated with melanogenesis. B16 melanoma cells stimulated by α-MSH alone showed high level of tyrosinase, TRP-1, and TRP-2 expressions, however, bieckol treatment suppressed the expression of with tyrosinase, TRP-1, and TRP-2 in a dose-dependent manner. Furthermore, bieckol inhibited the MITF protein expression in a dose-dependent manner, indicating that bieckol influenced the expression of melanogenic proteins at the translational level and inhibitory activity of bieckol was resulted from the down-regulation of tyrosinase not by direct inhibitory activity.

The various effects of whitening agents on the melanogenic enzymes have been reported that showed different inhibitory action. Hinokitiol has hypo-pigmentation effect through decreasing tyrosinase, TRP-1, and TRP-2 in protein level, and reducing the mRNA level of tyrosinase and MITF [27]. Linoleic acid affects the protein expression of tyrosinase, TRP-1, and TRP-2 without any expression of alterations of their mRNA level [28]. 4-n-Butylresorcinol inhibits the MITF protein

level and reduces tyrosinase activity [29]. Sphingosylphosphorylcholine inhibits MITF, tyrosinase, and TRP-2 in protein level, and reduces MITF and tyrosinase in mRNA level [30]. Pyrroloquinoline quinine has inhibitory effects of tyrosinase protein and mRNA expression, but it has no influence on TRP-1, and TRP-2 [3]. In this study, RT-PCR experiment was performed to determine whether the effects of bieckol on the melanogenic proteins were regulated at translation a transcriptional level. Bieckol down-regulated the melanogenic gene including tyrosinase, TRP-1 and TRP-2 at the transcriptional level with dose-dependent manner, indicating that down-regulation of MITF gene by bieckol affects the transcription of the melanogenic genes.

MITF is a member of the basic-helix-loop-helix-leucine zipper (bHLHLZ) family of transcription factors, and regulates melanogenesis and the development and differentiation of melanocytes. Mitogen-activated-protein-kinases (MAPKs) activated p90RSK induces phosphorylation of CREB Ser133 to up-regulate the MITF expression [31]. cAMP elevating agents stimulate the expression of MITF and binding of microphthalmia to the M-box and to the E-box, resulting in strong induction of the transcriptional activity of tyrosinase, TRP-1, and TRP-2 promoter [32-35]. On the other hand, phosphorylations of MITF at S73 by extracellular signal-regulated kinase (ERK) and at S409 by

Rsk-1 lead to ubiquitin-mediated degradation of MITF, and the ubiquitination of MITF down-regulate melanogenesis in melanocytes [36-38]. In addition, delayed ERK activation by TGF-β1 contributes to reduced melanin synthesis via MITF down-regulation [19] and delayed activation of ERK and Akt by C2-ceramide lead to the suppression of melanogenesis [39]. In this study, bieckol was also found to activate the ERK and Akt protein (1 hr) in α-MSH-stimulated B16 melanoma cells with delayed manner; peak ERK activation was observed at 6 hr and Akt activation was observed at 9 hr. Tyrosinase expression was also decreased at 6 and 9 hr, indicating that delayed activation of ERK and Akt by bieckol may influence on the ubiquitously degradation of MITF. These results suggest that bieckol may affect both on the transcription of MITF gene and on the degradation pathway of MITF to inhibit melanogenesis in α-MSH-stimulated B16 melanoma cells.

In summary, we demonstrated the depigmentation effect of bieckol on the α-MSH-stimulated B16 melanoma cells. Bieckol inhibited α-MSH-induced melanin production in B16 melanoma cells without alteration of proliferation. Our results show that bieckol inhibits tyrosinase, TRP-1, and TRP-2 gene transcription and subsequent protein expression through down-regulation of MITF.

Furthermore, phosphorylation of ERK and Akt regulate the melanogenic protein in α-MSH-B16 melanoma cells. Based on these results, we suggest that bieckol inhibits melanin synthesis in B16 melanoma cells with attenuation of melanogenic gene translation through down-regulation and degradation of MITF which regulated by ERK and Akt pathway, and we propose that bieckol may provide a beneficial effect for inhibitor of melanogenesis and it has potential for the development of functional cosmetics for whitening effects.

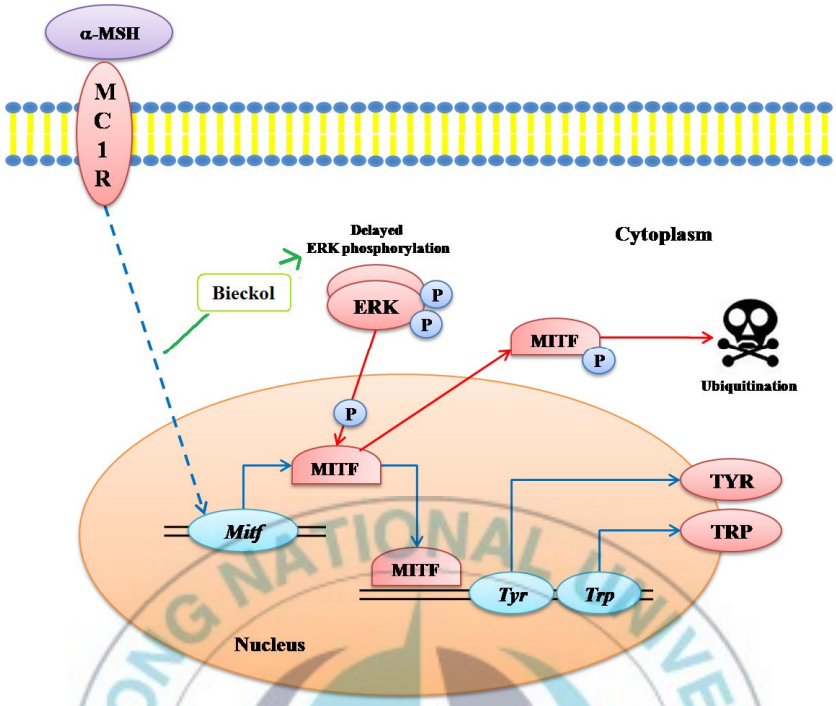

Fig. 9. The inhibitory effect of bieckol on melanogenesis via ERK and MITF transcriptional signal system.

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