Melanogenesis inhibitory activity of Korean Undaria pinnatifida in mouse B16 melanoma cells

Min-Jin KIM 1, Dong Sam KIM 1, Hun-Seok YOON 1, Wook Jae LEE 1, Nam Ho LEE 2, Chang-Gu HYUN 1,2
1 Jeju Biodiversity Research Institute (JBRI), Jeju Technopark, Jeju 699-943, Korea
2 Cosmetic Science Center, Faculty of Chemistry and Cosmetics, Jeju National University, Jeju 690-756, Korea

ABSTRACT
A number of seaweed species are used as traditional foods and medicine in different parts of the world, including Asian countries. However, very few data on the anti-melanogenic effect of seaweed have been published. Undaria pinnatifida (Dolmiyeok), a brown alga, is a traditional food in Jeju Island, the southern regions of the Korea peninsula. In this study, ethylacetate extracts of U. pinnatifida (UPE) were examined for their anti-melanogenic potentials. Our results supports the finding that UPE down-regulated melanin content in a dose-dependent pattern. To clarify the target of UPE action in melanogenesis, we performed Western blotting for tyrosinase and microphthalmia-associated transcription factor (MITF), which are key melanogenic enzymes. UPE inhibited tyrosinase and MITF expressions in a dose-dependent manner. These results indicate that treatment with UPE significantly inhibits the melanogenesis in B16 cells, and may be effective in the whitening agent for the skin.

KEY WORDS: Undaria pinnatifida; melanin; melanogenesis; tyrosinase; MITF

Introduction

There are many traditional foodstuffs in Korea. Undaria pinnatifida, called “Dolmiyeok or Miyeok” is one of them and is considered to be a healthy foodstuff in Korea. It is a very large brown alga, golden-brown in colour, related to the Laminaria species and other kelps. Adult specimens can grow to an overall length of between 1.5 and 3 m in less than a year – a rate of growth of up to one centimetre per day. Undaria pinnatifida is widely distributed along the coasts of Korea, Japan, China and Russia. Considering the traditional concept, several studies have focused on the beneficial effects of U. pinnatifida on anti-inflammatory (Khan et al., 2009; Yoo et al., 2012), antioxidant (Han et al., 2004; Hu et al., 2010; Moreira et al., 2010), antihypertensive (Suetsuma et al., 2004), antiviral (Thompson & Dragar, 2004; Hayashi et al., 2013), anticoagulating (Kim et al., 2010), and antiobesity (Okada et al., 2011) activities. However, the anti-melanogenic effect of U. pinnatifida extract has not been reported until now. Therefore, we conducted a detailed study to investigate the anti-melanogenic effects of U. pinnatifida extract in mouse B16 melanoma cells.

Melanin, produced by melanocytes in the basal layer of the epidermis, is principally responsible for skin colour and plays an important role in preventing skin damage caused by ultraviolet (UV) radiation. Melanin synthesis begins the conversion of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and then the oxidation of L-DOPA yields dopaquinone by tyrosinase enzyme, catalyzing the rate-limiting step for the melanin biosynthesis. This tyrosinase enzyme is involved in abnormal accumulation of melanin pigments, called hyperpigmentation (Wu et al., 2012; Chai et al., 2013). Therefore, tyrosinase inhibitors have been established as important constituents of whitening and depigmenting agents (Khan, 2012; Liang et al., 2013). However, side effects have been caused by the chemical inhibitors of tyrosinase. For example, arbutin caused a possible genotoxic effect and kojic acid, due to pigmented contact caused dermatitis. Thus the search for a safe and effective skin whitening agent is still a target of many studies in cosmetic industry (Yoon et al., 2010c; Kim et al., 2013).
A variety of seaweeds provide an interesting, largely unexplored source for the development of potential new drugs and skin-care cosmetics (Yang et al., 2010a; b; d; Yoon et al., 2010a; d). They have existed from antiquity to the present and have played significant roles in skin health and drug discovery, especially anti-inflammatory agents against skin diseases (Moon et al., 2011, Yang et al., 2010c; Yoon et al., 2010b). The seaweed extracts are also known to be inhibitors of melanin production, sometimes more potent than the classical inhibitors hydroquinone/ arbutin or kojic acid, and not associated with side effects. Therefore, the present study focused on whether the ethyl arbutin or kojic acid, and not associated with side effects.

Materials and methods

Materials and solvent extraction

_U. pinnatifida_ specimens were collected in April 2010 from Gapa Island, Korea. The specimen voucher (no. CSC-201) is deposited with Cosmetic Science Center, Department of Chemistry, Jeju National University, frozen and stored at –20°C until use. For extraction, the material was first ground into a fine powder and freeze-dried using a vacuum freeze-drier. The dried powder (90 g) was extracted with 80% ethanol (EtOH; 2 L) at room temperature for 24 h and then evaporated under vacuum. The evaporated EtOH extract (5 g) was suspended in water (1 L) and fractionated with ethyl acetate (EtOAc; 500 mL). The yield and recovery of EtOAc fractions were 0.6535 g and 13.1%, respectively.

Cell cultures

B16 murine melanoma cells were obtained from the Korean Cell Line Bank (Seoul). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% foetal bovine serum (FBS, Hyclone, Logan, UT, U.S.A.) and 1% penicillin-streptomycin (10,000 U/ml and 10,000 g/ml, respectively) in 5% CO2 at 37°C.

Cell viability assay

Cell viability assay was measured as described previously, with slight modification (Yoon et al., 2010c). Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded on 96-well plates, and drug treatment began 18 h after seeding. B16F10 murine melanoma cells were incubated with various concentrations of UPE for 72 h at 37°C in a humidified 95% air and 5% CO2 atmosphere. MTT (1 mg/mL in phosphate-buffer saline, PBS) was added to each well in a 1/10 volume of medium. Cells were incubated at 37°C for 4 h. Finally, the supernatant was removed and the formazan crystals were dissolved in DMSO. Absorbance was measured at 570 nm. Percent of cells showing cytotoxicity was determined relative to the control group.

Measurement of melanin contents

Extracellular melanin release was measured as described previously, with slight modification (Yoon et al., 2010c). Cells of the murine melanoma cell line, B16F10, plated at 1.0 × 10^5 cells/mL, were treated with α-MSH (50 nM) and then incubated with aliquots of UPE (3.125, 6.25 and 12.5 μg/mL) at 37°C for 72 h; the cells were then washed in ice-cold phosphate-buffered saline. Briefly, the samples were incubated at 80°C for 1 h in 1 mL of 1 N NaOH/10% DMSO and then vortexed to solubilize the melanin; the absorbance at 405 nm was then measured. Further, the melanin content was determined based on the absorbance/μg of protein in the extract from each cell. The protein concentration of the cells was determined by using a protein assay kit (Pierce, Rockford, IL, USA).

Determination of cellular tyrosinase activity

Cellular tyrosinase activity was measured by the method of Yen et al. with some modification (Yen et al., 2012). Briefly, the culture method for determining cellular tyrosinase assay was similar to that for determining melanin content. After treatment with different concentrations of UPE for 72 h, the cells were collected after treatment with trypsin-EDTA and centrifuged at 15,000 rpm for 15 min to obtain cell pellets. The pellet solutions were frozen and thawed twice and then centrifuged at 15,000 rpm for 15 min. We added 80 μL of the supernatant in a 96-well plate and mixed it with 20 μL of 0.2% L-DOPA. After incubation for 1 h, the optical densities were measured at 475 nm using a microplate spectrophotometer. The inhibitory activity of the UPE treated cells was presented as percentage against that of the untreated cells.

Measurement of tyrosinase and MITF in melanoma B16/F10 cells by Western blot

To determine the amount of tyrosinase and MITF protein, Western blotting analysis was performed. B16 melanoma cells that had been stimulated by α-MSH (50 nM) were treated with UPE (3.125, 6.25 and 12.5 μg/mL) for 3 days. After treatment, the cells were collected and lysed with cell lysis buffer [50 mM Tris–HCl (pH 6.8), 2% SDS, 6% mercaptoethanol, 1% glycerol]. Whole cell lysates (5 × 10^4 cells equivalents per lane) were separated by 7.5% SDS-polyacrylamide gel electrophoresis as described previously and transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% skimmed milk in phosphate-buffered saline containing 0.05% Tween 20. Tyrosinase and MITF bands were detected with rabbit polyclonal anti-tyrosinase antibody (dilution 1:1000) and rabbit polyclonal anti-MITF antibody (dilution 1:500), respectively, purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and then further incubated with horseradish peroxidase-conjugated anti-rabbit IgG antibody at a 1:5000 dilution. Bound antibodies were detected using an enhanced chemiluminescence kit (Amer sham Biosciences, Buckinghamshire, UK), following the manufacturer’s instructions. Loading control was assessed using anti-β-actin antibody. Positive bands were analysed using a gel image analysis instrument.
Statistical analysis
All data were obtained in triplicate and are represented as means ± standard error (SE). Significant differences between treatments were determined by Student’s t test in one-way analysis of variance (ANOVA).

Results and discussion
Melanin plays a crucial role in protecting the skin against harmful ultraviolet light, but overproduction and accumulation of melanin could create serious skin problems such as freckles, age pigment, and melasma. Thus, the inhibition of melanogenesis has been the focus on medicinal and cosmetic treatments for skin depigmenting and lightening. Therefore, this study focused on whether the ethyl acetate fraction from UPE inhibited melanin production and melanogenic protein expression on whether the ethyl acetate fraction from UPE inhibited this study focused medicinal and cosmetic treatments for skin depigmenting and lightening. Therefore, this study focused on whether the ethyl acetate fraction from UPE inhibited melanin production and melanogenic protein expression in mouse B16 melanoma cells. In the present study, the changes in the melanin contents in the cells treated with UPE were evaluated for anti-melanogenesis activity. The melanin contents of cells were significantly attenuated in one-way analysis of variance (ANOVA).

Figure 1

Figure 1. Inhibitory effect of UPE on melanin content (A) and cell viability (B) of B16F10 cells. B16F10 cells (2.0 × 10⁴ µg/mL) were pre-incubated for 18 h and the melanin content was assayed after incubation of the B16F10 cells treated with α-MSH (100 nM), melasolv (40 µM), and UPE (3.125, 6.25 and 12.5 µg/mL) for 72 h at 37 °C in a 5% CO2 atmosphere. The absorbance was measured at 405 nm with a spectrophotometer. The absorbance was measured at 405 nm with a spectrophotometer (Power Wave; Bio-tek, Winooski, VT). Values are the mean ± SEM of triplicate experiments. *p<0.05; **p<0.01.

Figure 2

Figure 2. Inhibitory effect on tyrosinase activity of UPE in B16F10 cells. B16F10 cells (2.0 × 10⁴ µg/mL) were pre-incubated for 18 h and tyrosinase activity was assessed after incubation of B16F10 cells treated with α-MSH (100 nM), melasolv (40 µM) and UPE (3.125, 6.25 and 12.5 µg/mL) for 72 h at 37 °C in a 5% CO2 atmosphere. Absorbance was measured at 405 nm with a ELISA. Values are the mean ± SEM of triplicate experiments. *p<0.05; **p<0.01.

Figure 3

Figure 3. Inhibitory effect of the UPE on the protein level related to melanogenic factors in B16F10 cells. B16F10 cells (1.0 × 10⁴ cells/mL) were pre-incubated for 18 h and were stimulated with α-MSH (100 nM) in the presence of melasolv (40 µM) and UPE (3.125, 6.25 and 12.5 µg/mL) for 24 h. The protein level was determined by immunoblotting.
Western blot analysis. As compared with the untreated control values, UPE-treated cells showed dose-dependent decreases in tyrosinase and MITF expression (Figure 3).

In summary, we investigated the anti-melanogenic effects of UPE and related melanogenic activity. The present results suggest that MITF protein levels are reduced by UPE. The hypopigmentation effect of UPE may be the result of down-regulation of MITF gene expression, which would then repress the protein and gene expressions of tyrosinase. Therefore we suggest that UPE can be a useful inhibitor of melanogenesis and has beneficial effects in the treatment of hyperpigmentation disorders such as epelis and oedema. However, the inhibitory mechanism of melanin production in B16 murine melanoma cells by UPE remains unclear. The investigation of the exact mechanisms and further in vivo experiments are needed to evaluate the possible use of UPE as a natural skin-whitening agent.

Acknowledgments

This research was financially supported by the Basic Science Research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science of Technology (2011-0007254). We are grateful to Jeju technopark for providing research circumstances for this study.

REFERENCES

This page contains a list of references cited in the text, but the specific references are not visible in the image provided. The references typically include details about the authors, publication year, title, journal, volume, issue, page numbers, and other bibliographic information. Each reference is formatted according to academic standards, providing credibility and traceability for the research claims made in the document. The references cited are likely related to the study of Undaria pinnatifida and its potential applications in skin care and health, given the context of the text and the focus on skin whitening and melanogenic effects.

In conclusion, the study’s findings suggest that UPE may be a promising natural skin-whitening agent due to its anti-melanogenic properties, with further research needed to fully understand its potential applications and mechanisms of action.

Kim WI, Koo YK, Jung MK, Moon HR, Kim SM, Synytsya A, Yun-Choi HS, Kim YS, Park JK, Park YI. (2010). Anticoagulating activities of low-molecular weight fuc-o-oligosaccharides prepared by enzymatic digestion of fucoidan from the spirophyll of Korean Undaria pinnatifida. Arch Pharm Res 33: 125–131.

Liang C, Lim JH, Kim SH, Kim DS. (2012). Dioscin: a synergistic tyrosinase inhibitor from the roots of Simalax china. Food Chem 134: 1146–1148.

Moon JY, Yang EJ, Kim SS, Kang JY, Kim GO, Lee NH, Hyun CG. (2011). Sosa quelpontensis phenylpropanoid derivative suppresses lipopolysaccharide-induced nitric oxide synthase and cyclo-oxygenase-2 expressions in RAW 264.7 cells. Yakugaku Zasshi 131: 961–967.

Moreira AS, González-Torres, L. Olivero-David R, Bastida S, Benedi J, Sánchez-Muniz FJ. (2010). Wakame and Nori in restructured meats included in cholesterol-enriched diets affect the antioxidant enzyme gene expressions and activities in Wistar rats. Plant Food Hum Nutr 65: 290–298.

Okada T, Mizuno Y, Sibayama S, Hosokawa M, Miyashita K. (2011). Antiocebity effects of Undaria lipid capsules prepared with scallop phospholipids. J Food Sci 76: H2–6.

Suetsuma K, Maekawa K, Chen JR. (2004). Antihypertensive effects of Undaria pinnatifida (wakame) peptide on blood pressure in spontaneously hypertensive rats. J Nutr Biochem 15: 267–272.

Thompson KD, Dragar C. (2004). Antiviral activity of Undaria pinnatifida against herpes simplex virus. Phytother Res 18: 551–555.

Wu X, Yin S, Zhong J, Ding W, Wan J, Xie Z. (2012). Mushroom tyrosinase inhibitors from Aloe barbadensis Miller. Fitoterapia 83: 1706–1711.

Yang EJ, Moon JY, Kim MJ, Kim DS, Kim CS, Lee WJ, Lee NH, Hyun CG. (2010). Inhibitory effect of Jeju endemic seaweeds on the production of pro-inflammatory mediators in mouse macrophage cell line RAW 264.7. J Jejung Univ Sci B 11: 315–322.

Yang EJ, Moon JY, Kim MJ, Kim DS, Lee WJ, Lee NH, Hyun CG. (2010). Anti-inflammatory effects of Petalonia binghamiae in LPS-induced macrophages is mediated by suppression of iNOS and COX-2. Int J Agri Biol 12: 754–758.

Yang EJ, Kim SS, Moon JY, Oh TH, Baik JS, Lee NH, Hyun CG. (2010). Inhibitory effects of Fortunella japonica var. margarita and Citrus sunki essential oils on nitric oxide production and skin pathogens. Acta Microbiol Immunol Hung 57: 15–27.

Yang EJ, Ham YM, Kim DS, Kim JY, Hong JP, Kim MJ, Kim GO, Lee NH, Hyun CG. (2010). Inhibitory effect of Ecklonia stolonifera isolated from Undaria pinnatifida in vitro. Int J Biol Macromol 46: 193–198.

Khan MF, Yoon WJ, Heo YJ, Park YI, Lee JT, Cho YW, Hong YK (2009). Anti-oxidative activity of sulfated polysaccharide fractions extracted from Undaria pinnatifida. Curr Med Chem 19: 2262–2272.

Kim SS, Hyun CG, Choi YH, Lee NH. (2013). Tyrosinase inhibitory activities of the compounds isolated from Neolitsea occulata (Blume) Koidz. J Enz Inhibit Med Chem 28(4): 685–689.

In conclusion, the study’s findings suggest that UPE may be a promising natural skin-whitening agent due to its anti-melanogenic properties, with further research needed to fully understand its potential applications and mechanisms of action.