Arch Pharm Res Vol 32, No 3, 367-373, 2009
DOI 10.1007/s12272-009-1309-8

Archives of Pharmacal Research
www.springer.com/12272

Inhibitory Effects of Arbutin on Melanin Biosynthesis of α-Melanocyte Stimulating Hormone-induced Hyperpigmentation in Cultured Brownish Guinea Pig Skin Tissues

Yu-Ji Lim[1], Eunjoo H. Lee[1], Tong Ho Kang[2], Sang Keun Ha[1], Myung Sook Oh[3], Seong Min Kim[4], Tae-Jin Yoon[4], Chulhun Kang[1], Ji-Ho Park[1], and Sun Yeou Kim[1,5]

[1]Department of Medical Science, Graduate School of East-West Medical Science, Kyung Hee University, Yongin 449-701, Korea, [2]Department of Oriental Medicinal Materials and Processing, College of Life Sciences, Kyung Hee University, Yongin 446-701, Korea, [3]Department of Oriental Pharmaceutical Science and Kyung Hee East-West Pharmaceutical Research Institute, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea, [4]Department of Dermatology, School of Medicine, Gyeongsang National University, Jinju 660-751, Korea, and [5]Skin Biotechnology Research Center, Kyung Hee University, Yongin 449-701, Korea

(Received June 12, 2008/Revised March 4, 2009/Accepted March 6, 2009)

Arbutin has been used as a whitening agent in cosmetic products. Melanin, the major pigment that gives color to skin, may be over-produced with sun exposure or in conditions such as melasma or hyperpigmentary diseases. Tyrosinase is a key enzyme that catalyzes melanin synthesis in melanocytes; therefore, inhibitors of the tyrosinase enzyme could be used for cosmetic skin whitening. A recent study has reported that arbutin decreases melanin biosynthesis through the inhibition of tyrosinase activity. However, this inhibitory mechanism of arbutin was not sufficiently demonstrated in skin tissue models. We found that arbutin both inhibits melanin production in B16 cells induced with α-MSH and decreases tyrosinase activity in a cell-free system. Furthermore, the hyperpigmentation effects of α-MSH were abrogated by the addition of arbutin to brownish guinea pig and human skin tissues. These results suggest that arbutin may be a useful agent for skin whitening.

Key words: α-MSH, Arbutin, Hyperpigmentation, Melanin, Melanocyte, Melanogenesis, Skin tissue culture, Tyrosinase, TRP-1, TRP-2

INTRODUCTION

Melanin plays an important role in phenotypic appearance, protection of the skin against ultraviolet (UV) rays, balance and auditory processing, absorption of toxic drugs and chemicals, and neurological development during embryogenesis (Jung et al., 2001; Virador et al., 1999; Yamaguchi et al., 2007). Skin colors depend on the amount, size, and type of melanins produced by melanocytes. Melanocytes are dendritic cells that exist in close contact with nearby epidermal keratinocytes. One of the roles of melanin is to protect the skin and underlying tissues from UV-induced skin injury. However, excessive melanin formation and the accumulation of melanin in the skin cause hyperpigmentation skin disorders such as melasma, freckles, and geriatric pigment spots (Ha et al., 2005; Kim et al., 2005).

In mammals, α-melanocyte-stimulating hormone (α-MSH) is required for hyperpigmentation. The α-MSH binds to its melanocortin-1-receptor (MC1R) and increases cyclic AMP (cAMP), which stimulates melanogenesis by activating microphthalmia-associated transcription factor (MITF), a melanocyte-specific transcription factor (Briganti et al., 2003; Kim et al., 2007; Ohguchi et al., 2005; Schwahn *et al.*, 2001). Melanin synthesis is mainly regulated by tyrosinase, tyrosinase-related protein-1 (TRP-1), and TRP-2. Tyrosinase is a bifunctional enzyme that

Correspondence to: Ji-Ho Park and Sun Yeou Kim, Department of Medical Science, Graduate School of East-West Medical Science, Kyung Hee University, Yongin 449-701, Korea
Tel: 82-31-201-2177, Fax: 82-31-205-8962
E-mail: sunnykim@khu.ac.kr, jihopark@khu.ac.kr

plays a significant role in the modulation of melanin production, first by catalyzing the hydroxylation of tyrosine to DOPA and second by catalyzing the oxidation of DOPA to DOPA-quinone. TRP-2, which functions as a DOPAchrome tautomerase, catalyzes the rearrangement of DOPAchrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA); TRP-1 oxidizes DHICA to a carboxylated indole-quinone.

In mammals, two major types of melanin are formed: black/brown eumelanins and yellow/red pheomelanins. Tyrosinase is required by both eumelanin and pheomelanin. In contrast, TRP-1 and TRP-2 are more important for eumelanin biosynthesis than for pheomelanin biosynthesis (Kim et al., 2005; Tsuji-Naito et al., 2007; Zhong et al., 2006). Tyrosinase inhibitors have potential for the treatment of hyperpigmentary disorders and can be used as skin-whitening agents in the cosmetic industry (Virador et al., 1999). Arbutin has a hydroquinone moiety similar to that of hydroquinone, a tyrosinase inhibitor. It was previously reported that arbutin exhibits dose-dependent inhibitory effects on tyrosinase activity in B16 and HMV- melanoma cells (Sugimoto et al., 2003; Sugimoto et al., 2004; Akiu et al., 1991). Despite its use as a whitening agent, the anti-melanogenesis mechanism of arbutin is not fully understood at the molecular level (Hori et al., 2004).

The culture of skin tissue such as guinea pig or human skin is a valuable technique that can be used for investigations of skin biology, cutaneous pharmacology, and skin diseases (Tammi et al., 1979). The cultured skin tissue system is more physiologically relevant than artificial skin equivalents that contain cultured melanocytes and keratinocytes for tests of effects on the pigmentation of bioactive melanogenic compounds. Furthermore, guinea pig skin serves as an excellent pigmentation model because it is similar to human skin (Tobiishi et al., 2004; Yoshida et al., 2002). To date, the inhibitory mechanism of arbutin in brownish guinea pig skin and human skin models has not been examined. Therefore, we examined the inhibitory effects of arbutin on melanin biosynthesis in α-MSH-stimulated B16 melanoma cells, cultured brownish guinea pig skin tissue, and human skin tissue.

MATERIALS AND METHODS

Cell culture and treatment

B16 cells (American Type Culture Collection [ATCC]) were cultured in DMEM with 10% fetal bovine serum and penicillin/streptomycin in air containing 5% CO_2 at 37°C. During incubation, the medium was changed every 2 days. A stock solution of arbutin was made at a concentration of 10 mM in phosphate-buffered saline (PBS) and was added to the cell culture at final concentrations of 10, 100, 250, 500, and 1000 μM. After 72 h of treatment with the samples, the cells were harvested and used for various assays.

Melanin content assay

Melanin content was determined according to the modified methods of Hosoi et al. (1985). B16 cells were cultured at 1×10^5 cells/well with 10 nM α-MSH in six-well plates. After 24 h, the cells were treated with various concentrations of arbutin (10-1000 μM) for 72 h. After washing with PBS, the cells were harvested by trypsinization. The cell pellet was solubilized in 200 μL of 1 N NaOH containing 10% DMSO at 80°C for 1 h. The absorbance of each well was measured at 405 nm using a spectrophotometer.

Effect of arbutin on cell viability

The cell proliferation assay was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). B16 cells were cultured at 4×10^3 cells/well with 10 nM α-MSH in 96-well plates. After 24 h, cells were treated with various concentrations of arbutin (10-1000 μM) for 72 h. At the end of the incubation, 50 μL of MTT solution (1 mg/mL in PBS) was added to each well. After incubation at 37°C for 2 h, the medium was gently removed and 150 μL of 0.04 N HCl/isopropanol was added. The absorbance of each well was measured at 570 nm using a spectrophotometer.

Mushroom tyrosinase activity assay

Mushroom tyrosinase was purchased from Sigma Chemical (St. Louis, MO, USA). Various concentrations of test material (10-1000 μM) were dissolved in PBS. We added to each well of a 96-well microplate, 120 μL of 8.3 mM L-dopa (dissolved in 80 mM phosphate buffer, pH 6.8) and 40 μL of either the same buffer or a test sample; 40 μL of mushroom tyrosinase (125 U dissolved in 80 mM phosphate buffer, pH 6.8) was then mixed into the solution. The amount of dopachrome in the reaction mixture was measured after incubation at 37°C for 30 min. The inhibitory activity of the sample was determined based on the optical density at 490 nm using a spectrophotometer.

Western blotting

Changes in the levels of melanin formation-related proteins, including tyrosinase and TRP-1, in B16 cells following treatment with 10, 100, and 250 μM arbutin were evaluated by Western blotting. After washing with PBS, cells were lysed in ice-cold RIPA buffer containing protease inhibitors and centrifuged at 13,000 rpm for 20 min at 4°C. The supernatant was collected and assayed for protein concentration using the DC protein assay kit (Bio-rad, Hercules, CA, USA). Lysates containing 20 μL of protein were separated by SDS-PAGE with 10% resolving and 3% acrylamide stacking gel and were transferred to nitrocellulose sheets. Blocking was performed in Tris-buffer saline containing 5% skim milk powder and 0.1% Tween-20. The primary antibodies used for Western blotting included mouse anti-α-tubulin antibody (Sigma, MO, USA), goat anti-tyrosinase, and goat anti-TRP-1 (Santa Cruz Biotechnology, CA, USA).

Skin specimen organ culture

Human skin tissues were obtained from skin surgeries of 57-year-old women (n = 8). Female brownish guinea pigs (350-600 g) were used in the pigmentation study. Organ culture methods were used with minor modifications for whole skin surviving for 5 days, so that the structure of the epidermis and dermis closely resembled that of skin *in vivo* (Tammi et al., 1979). Hair was shaved from the dorsal skin of guinea pigs using an electric shaver, and a 4-mm punch biopsy was taken. Subcutaneous fat and lower dermis were carefully removed under a stereomicroscope using a surgical scalpel. Skin samples were placed on culture inserts (polytetrafluorethylene membranes, 0.4-μm filter pore size; Millipore, Bedford, MA, USA) with the uppermost epithelium facing the air-liquid interface. The inserts were placed in six-well plates for 5 days at 32°C in a humidified incubator with 5% CO_2. Organ culture was performed with DMEM containing 10% bovine calf serum and penicillin/streptomycin (100 IU/50 μg/mL). The medium was changed daily.

Arbutin treatment

Each of the skin biopsies was stabilized and treated with and α-MSH for 24 h. The α-MSH induced an increase in melanin production. After 24 h, skin tissues were treated with 10 mM arbutin for 72 h. After 3 days of survival *ex vivo*, the skin fragments were removed from the culture inserts and the effect of arbutin on the brownish guinea pig skin tissues was examined histologically.

Histological study

Skin biopsy specimens were fixed in 4% paraformaldehyde solution at 4°C for 24 h. The tissue was then dehydrated and embedded in paraffin according to standard procedures. Serial sections of 5 μm thickness were obtained. Melanocytes and melanin were visualized using Fontana-Masson staining (Grabe, 1968).

RESULTS

Melanogenesis of B16 cells induced with α-MSH

Melanogenesis was initiated by the addition of 1 or 10 nM α-MSH and was assessed by the determination of the intracellular melanin content. After stimulation with α-MSH for 24 h, the melanin content increased in a dose-dependent manner, reaching maximum at 10 nM α-MSH (Fig. 1).

Effect of arbutin on cell viability and the melanin content in B16 cells

The viability of B16 cells after arbutin treatment was examined using the MTT assay. Arbutin was not significantly cytotoxic to B16 cells at concentrations of 10-250 μM, although it had some cytotoxicity in higher doses (Fig. 2A). To investigate the effect of arbutin on melanogenesis, B16 cells were cultured in the presence of 10-1000 μM arbutin. Arbutin reduced melanin production in a dose-dependent manner (Fig. 2). Upon exposure to α-MSH alone, the melanin content of cells increased by approximately 23%. Arbutin exhibited dose-dependent inhibitory effects of 66% inhibition at

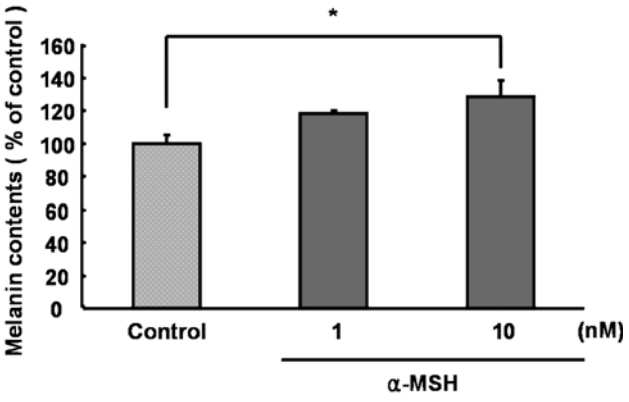

Fig. 1. Melanogenesis in B16 melanoma cells induced by α-MSH. The cells were treated with 1 and 10 nM α-MSH for 24 h, and the melanin content was determined as described in Materials and Methods. Data represent the mean ± SD of five experiments; *P < 0.05, compared with the control.

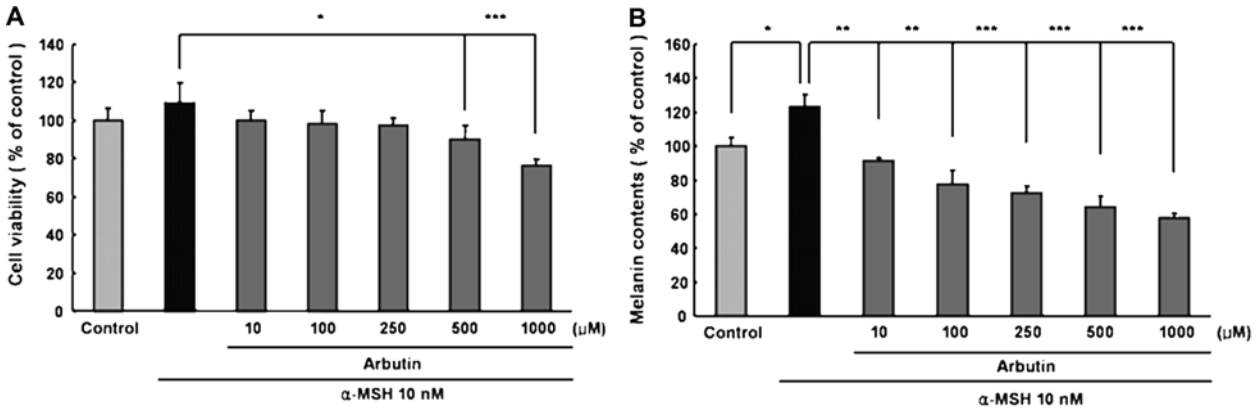

Fig. 2. Effects of arbutin on the cell viability and melanin content of B16 cells. A. Cells were treated with various doses of arbutin (10-1000 μM) and were examined using the MTT assay. Data represent the mean ± SD of five experiments. B. B16 cells were incubated with various concentrations of arbutin (10-1000 μM) for 72 h, and the melanin content was then determined. Data represent the mean ± SD of five experiments; *P < 0.05, **P < 0.01, ***P < 0.001, compared with the control.

1000 μM, 59% at 500 μM, 51% at 250 μM, 46% at 100 μM, and 32% at 10 μM (Fig. 2B).

Effect of arbutin on mushroom tyrosinase activity and the protein expression of melanogenic enzymes

Arbutin was investigated for inhibitory activity toward mushroom tyrosinase. Arbutin inhibited the DOPA oxidase activity of mushroom tyrosinase in a dose-dependent manner (Fig. 3A). Up to 40% inhibition was observed at 1000 μM arbutin compared with the control (Fig. 3A). The protein expression levels of tyrosinase and TRP-1 were examined using Western blots. The α-MSH increases melano-

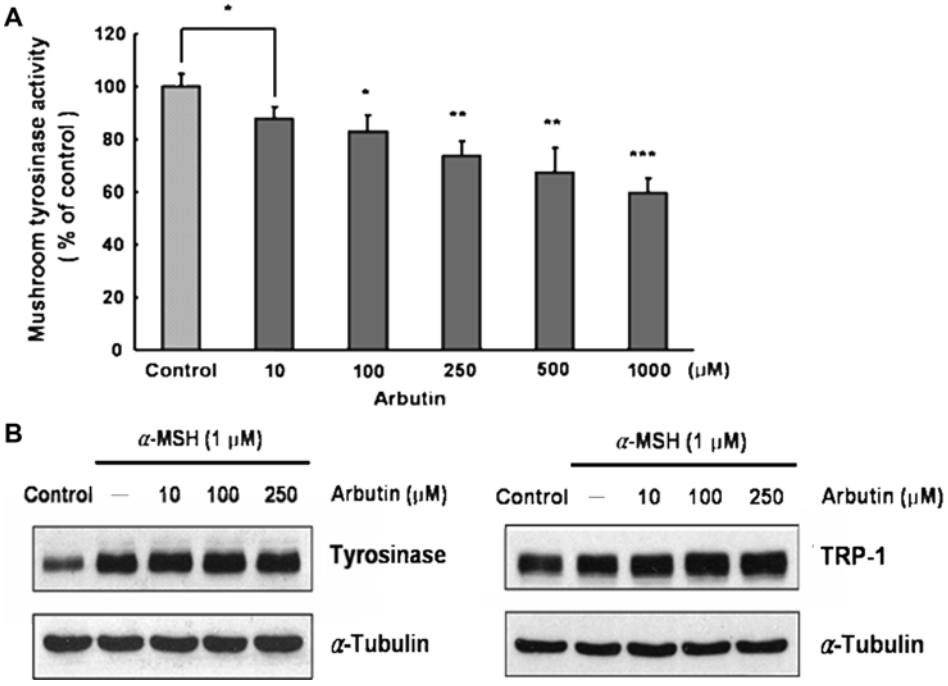

Fig. 3. Effects of arbutin on mushroom tyrosinase activity and the protein expression of melanogenic enzymes. A. Mushroom tyrosinase activity was determined using L-DOPA as the substrate in a cell-free system. Data represent the mean ± SD of five experiments; *P < 0.05, **P < 0.01, ***P < 0.001, compared with the control. B. B16 cells were cultured with 1 μM α-MSH and treated with 50, 100, or 250 μM arbutin for 72 h. Western blots were performed for whole-cell lysates using antibodies against tyrosinase and TRP-1. Equal protein loading was confirmed using anti-α-tubulin antibody.

genic enzyme protein levels in melanocytes (Ohguchi et al., 2005). We confirmed that 1 μM α-MSH stimulated tyrosinase and TRP-1 gene expression in B16 cells. Otherwise, no significant differences were observed in tyrosinase and TRP-1 protein expression between the arbutin-treated and untreated B16 cells (Fig. 3B).

Melanin content visualized by Fontana-Masson staining of brownish guinea pig and human skin

The distribution of melanin in the skin is a critical factor in determining the constitutive skin color and photoprotective efficacy (Sturm et al., 1998). Melanin transfer and distribution can be modulated by the melanogenesis stimulator α-MSH (Thody, 1999). We used Fontana-Masson staining of the skin after 24 h of incubation with various concentrations of α-MSH to demonstrate that the melanin distribution was increased compared to the control (Fig. 4A). In contrast, using a similar analysis, we treated skin with the melanogenesis inhibitor arbutin for 72 h. The melanin content was decreased in epidermis that was exposed to 10 mM arbutin (Fig. 4B). We thus demonstrated a decrease in melanin content in human skin specimens following treatment with arbutin. Using the same tissue culture methods, we stimulated melanogenesis with α-MSH for 24 h and then treated the skin with 10 mM arbutin for 72 h. The proportion of pigmented cells with melanin content decreased significantly under this condition (Fig. 4C).

DISCUSSION

Melanin is an exclusive pigmented biopolymer that is synthesized by specialized cells known as melanocytes. UV radiation regulates melanogenesis

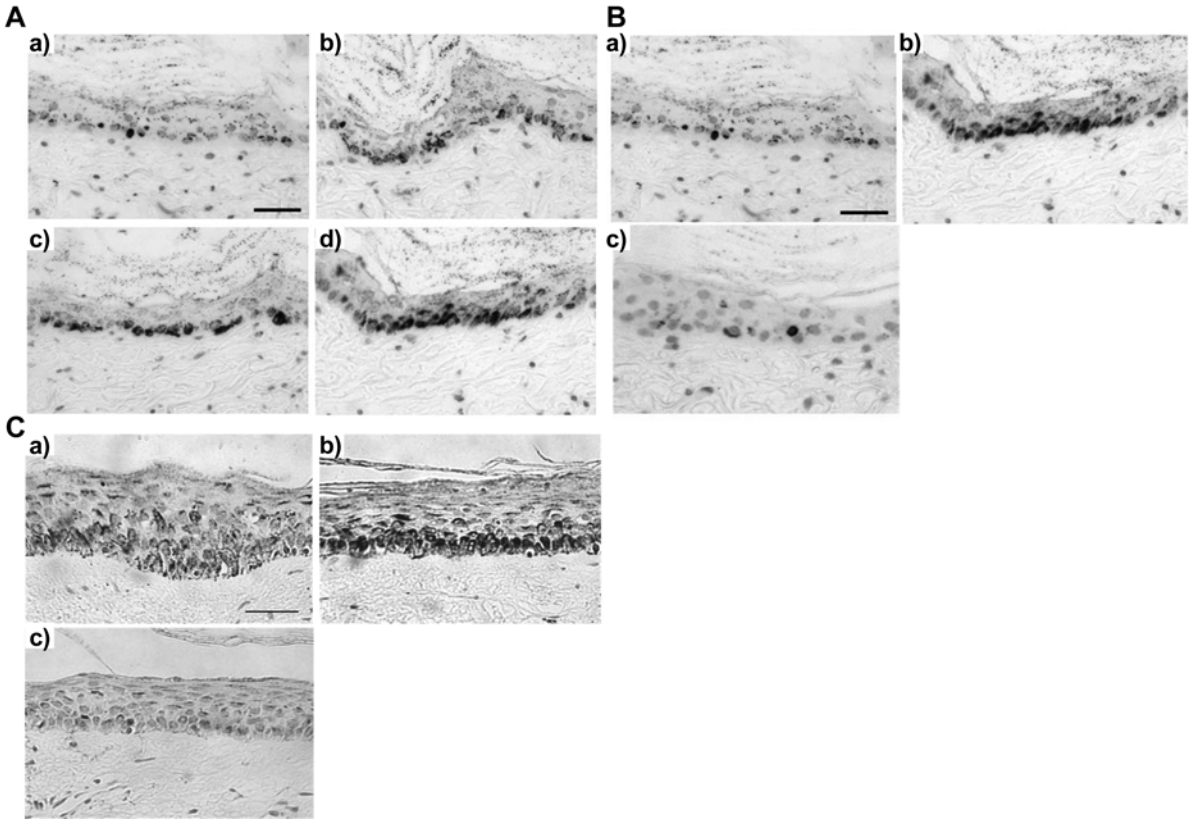

Fig. 4. Melanin content visualized by Fontana-Masson staining of brownish guinea pig and human skin. A. The morphological distribution of melanin in brownish guinea pig skin as visualized using Fontana-Masson staining. Pigmented skin was treated for 24 h with 100, 200, or 500 nM of α-MSH. a) control; b) 100 nM α-MSH; c) 200 nM α-MSH; d) 500 nM α-MSH. Scale bar = 20 μm. B. The melanin content was visualized using Fontana-Masson staining of brownish guinea pig skin. The skin was treated with α-MSH for 24 h and then with arbutin for 72 h. a) control; b) 500 nM α-MSH treatment; c) 500 nM α-MSH and 1 mM arbutin treatment. Scale bar = 20 μm. C. The melanin content was visualized using Fontana-Masson staining of human skin. The skin was treated with α-MSH for 24 h and then with arbutin for 72 h. a) control; b) 500 nM α-MSH treatment; c) 500 nM α-MSH and 1 mM arbutin treatment. Scale bar = 20 μm.

directly by increasing tyrosinase expression and activity in melanocytes or indirectly through the release of keratinocyte-derived factor; this induces the subsequent accumulation of melanosomes in melanocytes (Kim et al., 2005). Adrenocorticotropic hormone (ACTH) and α-MSH enhance melanocyte proliferation, melanogenesis, and melanocyte dendrite formation (Sulaimon & Kitchell, 2003). The α-MSH plays numerous roles in the skin. By activating the MC1 receptor on melanocytes, α-MSH stimulates melanogenesis; α-MSH acts specifically to stimulate eumelanin synthesis, rather than producing large increases in melanin production. In humans, pigmentation plays an important cosmetic role, which can be compromised in certain hyperpigmentary skin conditions and/or in conditions such as melasma, age spotting, and post-inflammatory hyperpigmentation (Usuki et al., 2003; Virador et al., 1999). The regulation of human skin pigmentation has been a long-sought-after aim for cosmetic and pharmaceutical applications. Many whitening cosmetics and medicines such as hydroquinones, kojic acid, and their derivatives have been developed for cosmetic skin-whitening purposes. However, these products have not been fully satisfying because they produce poor results or side effects. Arbutin has been widely used as a whitening agent, along with tyrosinase inhibitor. It has been reported that arbutin reduces cellular tyrosinase activity in a human melanocyte culture and in human melanoma cells at non-cytotoxic concentrations and that it does not affect tyrosinase mRNA expression levels (Maeda and Fukuda, 1996; Nakajima et al., 1998). Moreover, Western blots indicated that there was no change in protein content or the molecular size of melanogenic enzymes after arbutin treatment. However, the effects of arbutin had not been determined in cultured brownish guinea pig skin or a human skin model. Tyrosinase (monophenol, 3,4-dihydroxyphenyalanine:oxygen oxidoreductase, EC 1.14.18.1) is synthesized and glycosylated in the endoplasmic reticulum and Golgi apparatus. The enzyme is continuously transported to melanosomes, where it participates in melanogenesis (Jimenez et al., 1988). We demonstrated that the addition of arbutin blocks and inhibits α-MSH-stimulated melanogenesis in B16 melanoma cells, brownish guinea pig, and human skin tissue. These observations imply that the depigmenting effect of arbutin may be highly effective in both cell culture and *ex vivo* skin models. Skin tissue culture has the advantage of allowing extensive testing of various bioactivities with minimal expense and effort. This *ex vivo* tissue culture is an appropriate experimental model with which to study the events leading to pigmentation in the epidermis, as are other *ex vivo* models (Boisnic et al., 2005). If a reduction in melanin distribution within skin tissue is observed following the use of a whitening substance, the substance may be valuable for use in the development of whitening cosmetics and medicines. We did not attempt to measure whether arbutin regulates the glycosylation pattern of tyrosinase. Furthermore, the detailed signal transduction pathway of the arbutin-mediated inhibitory effect on melanogenesis necessitates further study. In conclusion, our results indicate that arbutin should be recognized as a useful depigmenting compound for skin lightening in the cosmetic industry.

ACKNOWLEDGEMENTS

This work was supported by Skin Biotechnology Research Center/KISTEP and the SRC/ERC program of MOST/KOSEF(R11-2002-103).

REFERENCES

Akiu, S., Suzuki, Y., Asahara, T., Fujinuma, Y., and Fukuda, M., Inhibitory effect of arbutin on melanogenesis-biochemical study using cultured B16 melanoma cells. *Nippon. Hifuka. Gakkai. Zasshi.*, 101, 609-613 (1991).

Boisnic, S., Branchet-Gumila, M. C., Nocera, T., and Verriere, F., RALGA (Diacneal) decreases melanin content in a human skin model. *Dermatology.,* 210 Suppl 1, 35-38 (2005).

Briganti, S., Camera, E., and Picardo, M., Chemical and instrumental approaches to treat hyperpigmentation. *Pigment. Cell. Res.*, 16, 101-110 (2003).

Grabe, M., *Techniques histologiques.* Masson, Paris, (1968).

Ha, S. K., Koketsu, M., Lee, K., Choi, S. Y., Park, J. H., Ishihara, H., and Kim, S. Y., Inhibition of tyrosinase activity by N,N-unsubstituted selenourea derivatives. *Biol. Pharm. Bull.,* 28, 838-840 (2005).

Hori, I., Nihei, K., and Kubo, I., Structural criteria for depigmenting mechanism of arbutin. *Phytother. Res.*, 18, 475-479 (2004).

Hosoi, J., Abe, E., Suda, T., and Kuroki, T., Regulation of melanin synthesis of B16 mouse melanoma cells by 1 alpha, 25-dihydroxyvitamin D3 and retinoic acid. *Cancer. Res.*, 45, 1474-1478 (1985).

Jimenez, M., Kameyama, K., Maloy, W. L., Tomita, Y., and Hearing, V. J., Mammalian tyrosinase: biosynthesis, processing, and modulation by melanocyte-stimulating hormone. *Proc. Natl. Acad. Sci USA.,* 85, 3830-3834 (1988).

Jung, G. D., Yang, J. Y., Song, E. S., and Par, J. W.,

Stimulation of melanogenesis by glycyrrhizin in B16 melanoma cells. *Exp. Mol. Med.,* 33, 131-135 (2001).

Kim, D. S., Jeong, Y. M., Park, I. K. et al., A new 2-imino-1,3-thiazoline derivative, KHG22394, inhibits melanin synthesis in mouse B16 melanoma cells. *Biol. Pharm. Bull.*, 30, 180-183 (2007).

Kim, Y. J., No, J. K., Lee, J. H., and Chung, H. Y., 4,4'-Dihydroxybiphenyl as a new potent tyrosinase inhibitor. *Biol. Pharm. Bull.,* 28, 323-327 (2005).

Maeda, K. and Fukuda, M., Arbutin: mechanism of its depigmenting action in human melanocyte culture. *J. Pharmacol. Exp. Ther.* 276, 765-769 (1996).

Nakajima, M., Shinoda, I., Fukuwatari, Y., and Hayasawa, H., Arbutin increases the pigmentation of cultured human melanocytes through mechanisms other than the induction of tyrosinase activity. *Pigment. Cell. Res.,* 11, 12-17 (1998).

Ohguchi, K., Akao, Y., and Nozawa, Y., Involvement of calpain in melanogenesis of mouse B16 melanoma cells. *Mol. Cell. Biochem.*, 275, 103-107 (2005).

Schwahn, D. J., Xu, W., Herrin, A. B., Bales, E. S., and Medrano, E. E., Tyrosine levels regulate the melanogenic response to alpha-melanocyte-stimulating hormone in human melanocytes: Implications for pigmentation and proliferation. *Pigment. Cell. Res.,* 14, 32-39 (2001).

Sturm, R. A., Box, N. F., and Ramsay, M., Human pigmentation genetics: the difference is only skin deep. *Bioessays.,* 20, 712-721 (1998).

Sugimoto, K., Nishimura, T., Nomura, K., Sugimoto, K., and Kuriki, T., Syntheses of arbutin-alpha-glycosides and a comparison of their inhibitory effects with those of alpha-arbutin and arbutin on human tyrosinase. *Chem. Pharm. Bull (Tokyo).,* 51, 798-801 (2003).

Sugimoto, K., Nishimura, T., Nomura, K., Sugimoto, K., and Kuriki, T., Inhibitory effects of alpha-arbutin on melanin synthesis in cultured human melanoma cells and a three-dimensional human skin model. *Biol. Pharm. Bull.,* 27, 510-514 (2004).

Sulaimon, S. S. and Kitchell, B. E., The biology of melanocytes. *Vet. Dermatol.* 14, 57-65 (2003).

Tammi, R., Jansen, C. T., and Santti, R., Histometric analysis of human skin in organ culture. *J. Invest. Dermatol.*, 73, 138-140 (1979).

Thody, A. J., alpha-MSH and the regulation of melanocyte function. *Ann. N Y Acad. Sci.*, 885, 217-229 (1999).

Tobiishi, M., Haratake, A., Kaminaga, H., Nakahara, M., Komiya, A., Koishikawa, H., Uchiwa, H., Kawa, Y., and Mizoguchi, M., Pigmentation in intrinsically aged skin of A1 guinea pigs. *Pigment. Cell. Res.* 17, 651-658 (2004).

Tsuji-Naito, K., Hatani, T., Okada, T., and Tehara, T., Modulating effects of a novel skin-lightening agent, alpha-lipoic acid derivative, on melanin production by the formation of DOPA conjugate products. *Bioorg. Med. Chem.*, 15, 1967-1975 (2007).

Usuki, A., Ohashi, A., Sato, H., Ochiai, Y., Ichihashi, M., and Funasaka, Y., The inhibitory effect of glycolic acid and lactic acid on melanin synthesis in melanoma cells. *Exp. Dermatol.*, 12 Suppl 2, 43-50 (2003).

Virador, V. M., Kobayashi, N., Matsunaga, J., and Hearing, V. J., A standardized protocol for assessing regulators of pigmentation. *Anal. Biochem.,* 270, 207-219 (1999).

Yamaguchi, Y., Brenner, M., and Hearing, V. J., The regulation of skin pigmentation. *J. Biol. Chem.* 282, 27557-27561 (2007).

Yoshida, M., Hirotsu, S., Nakahara, M., Uchiwa, H., and Tomita, Y., Histamine is involved in ultraviolet B-induced pigmentation of guinea pig skin. *J. Invest. Dermatol.*, 118, 255-260 (2002).

Zhong, S., Wu, Y., Soo-Mi, A., Zhao, J., Wang, K., Yang, S., Jae-Ho, Y., and Zhu, X., Depigmentation of melanocytes by the treatment of extracts from traditional Chinese herbs: a cell culture assay. *Biol. Pharm. Bull.*, 29, 1947-1951 (2006).