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Research Article

Antimelanogenesis and skin-protective activities of *Panax ginseng* calyx ethanol extract

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ABSTRACT

Background: The antioxidant effects of *Panax ginseng* have been reported in several articles; however, little is known about the antimelanogenesis effect, skin-protective effect, and cellular mechanism of *Panax ginseng*, especially of *P. ginseng* calyx. To understand how an ethanol extract of *P. ginseng* berry calyx (Pg-C-EE) exerts skin-protective effects, we studied its activities in activated melanocytes and reactive oxygen species (ROS)–induced keratinocytes.

Methods: To confirm the antimelanogenesis effect of Pg-C-EE, we analyzed melanin synthesis and secretion and messenger RNA and protein expression levels of related genes. Ultraviolet B (UVB) and hydrogen peroxide (H_2O_2) were used to induce cell damage by ROS generation. To examine whether this damage is inhibited by Pg-C-EE, we performed cell viability assays and gene expression and transcriptional activation analyses.

Results: Pg-C-EE inhibited melanin synthesis and secretion by blocking activator protein 1 regulatory enzymes such as p38, extracellular signal-regulated kinases (ERKs), and cyclic adenosine monophosphate response element–binding protein. Pg-C-EE also suppressed ROS generation induced by H_2O_2 and UVB. Treatment with Pg-C-EE decreased the expression of matrix metalloproteinases, mitogen-activated protein kinases, and hyaluronidases and increased the cell survival rate.

Conclusion: These results suggest that Pg-C-EE may have antimelanogenesis properties and skin-protective properties through regulation of activator protein 1 and cyclic adenosine monophosphate response element–binding protein signaling. Pg-C-EE may be used as a skin-improving agent, with moisture retention and whitening effects.

1. Introduction

Our body surfaces are defended by epithelia, which impose a physical barrier between the internal milieu and the external world that contains pathogens. Epithelial cells are held by tight junctions, which effectively form a seal against the external environment including infection with pathogens and excessive water loss [1]. In humans, the skin is the largest organ of the integumentary system. The skin has three layers of ectodermal tissue: epidermis, dermis, and subcutaneous tissue (hypodermis). The epidermis layer is the outer layer of the skin and contains merkel cells, keratinocytes, melanocytes, and langerhans cells. This layer plays an important role in maintaining the body temperature. The dermis layer, located beneath the epidermis layer, contains many nerve endings, hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels, and blood vessels. The subcutaneous tissue lies beneath the

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dermis layer and consists of loose connective tissue, adipose tissue, elastin, fibroblasts, macrophages, and adipocytes [2]. Melanin in the basal layer of the epidermis plays an important role in the determination of skin color and melanogenesis. Melanin is produced within the skin in cells called melanocytes. Although melanogenesis is not fully understood, substances that stimulate melanin production are known, such as melanocyte-stimulating hormone, forskolin, cholera toxin, isobutylmethylxanthine, diacylglycerol analogs, ultraviolet (UV) irradiation, and vitamin D metabolites [2], which trigger melanogenesis and severe diseases such as Cushing's disease and melanocytic tumor. Tyrosinase, a melanogenesis-related enzyme, is an oxidase that controls melanin production. This enzyme is involved in the hydroxylation of a monophenol and the conversion of an o-diphenol to the corresponding o-quinone, which in turn is converted to melanin through several reactions. Tyrosinase is found inside melanosomes together with tyrosinase-related protein-1 (TRP-1) and tyrosinase-related protein-2 (TRP-2). Genes associated with melanogenesis include microphthalmia-associated transcription factor (MITF), melanophilin (MLPH), ras-related protein Rab27a, and myosin-5A (Myo5A). Tyrosinase, TRP-1, and TRP-2 are involved in melanin synthesis, whereas MLPH, Rab27a, and Myo5A play a role in melanin secretion [3–5].

UV irradiation induces photoaging and reactive oxygen species (ROS) generation in addition to melanogenesis. UV irradiation damages the human skin and induces the synthesis of matrix metalloproteinases (MMPs) in fibroblasts and keratinocytes. MMPs mainly play a role in augmentation of dermal collagen degradation during UV exposure. Increased expression of MMPs causes severe problems including dermal photoaging and carcinogenesis [6,7]. Types of MMPs expressed in skin cells are MMP-1 (interstitial collagenase), MMP-2 and -9 (gelatinase-A and -B), and MMP-3 (stromelysin 1) [8]. In addition, exogenous ROS can be produced by exposure to hydrogen peroxide (H_2O_2) and UV irradiation. ROS stimulate the transcription of genes encoding proinflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, and cyclooxygenase-2 (COX-2) as well as MMPs via mitogen-activated protein kinases (MAPKs) [9,10]. In particular, H_2O_2 regulates heme oxygenase 1 and nuclear factor 2 via oxidative stress and damage [11]. As mentioned previously, moisture retention plays important roles in the skin. Natural moisture factors (NMF) such as hyaluronic acid (HA) are involved in skin moisture retention and are an essential component of the extracellular matrix (ECM). Genes related to NMF production are filaggrin (FLG, epidermal barrier protein), transglutaminase-1, and hyaluronic acid synthase (HAS)-1, -2, and -3 [12]. In contrast to HAS genes, hyaluronidase (HYAL)-1, -2, -3, and -4 catalyze the degradation of HA. Hyaluronidase is known to be overexpressed during UV photoaging [13].

Panax ginseng has been used as an herbal medicine in Korea, China, and Japan for a very long time. The value of ginseng as a medicinal herb is attributed to an effective group of compounds called ginsenosides. Ginsenosides consist of protopanaxadiol and protopanaxatriol, which have known pharmacological features and physiological activities including antioxidant, immunomodulation, antidiabetic, and anticancer effects [14,15]. *P. ginseng* calyx is the peduncle between the berry and root of ginseng. Ginseng calyx is not usually used in ginseng industries; therefore, it is mostly removed in large quantities during the process of harvest of ginseng roots. Nonetheless, because various kinds of ginsenosides were found to be highly existing in the ginseng calyx part, its industrial application has been proposed. Indeed, previous studies have shown that *P. ginseng* calyx contains the highest amount of G-Re (6%) among protopanaxatriol compounds [16] with skin barrier functions [17]. The antioxidant effects of *P. ginseng* have been reported in several articles [18,19]. However, little is known about its antimelanogenesis and skin-protective effects or the cellular mechanism, especially for *P. ginseng* calyx.

2. Materials and methods

2.1. Materials

B16F10, HaCaT, and HEK293 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, phosphate-buffered saline (PBS), and penicillin-streptomycin were purchased from HyClone Laboratories Inc., (Logan, UT, USA). 3-(4-5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco (Brisbane, Australia). α-Melanocyte-stimulating hormone (a-MSH), arbutin, L-dopa-(phenyl-d3) (L-DOPA), mushroom tyrosinase (tyrosinase), kojic acid, retinol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, phorbol 12-myristate 13-acetate (PMA), forskolin from *Coleus forskohlii* (forskolin), and polyethylenimine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2; 35%) was purchased from JUNSEI (Chuo-ku, Tokyo, Japan). SB203580 (SB), SP600125 (SP), and U0126 were purchased from Calbiochem (La Jolla, CA, USA). TRIzol reagent was purchased from MRCgene (OH, USA), and the cDNA synthesis kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The primer sets (forward and reverse) for polymerase chain reaction (PCR) were synthesized by Macrogen (Seoul, Korea), and PCR premix was purchased from Bio-D Inc. (Seoul, Korea). The luciferase assay system was purchased from Promega (Madison, WI, USA). Polyvinylidenedifluoride membrane was from Merck Millipore (Billerica, MA, USA). Antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA) and Abcam (Cambridge, MA, USA). Pg-C-EE was prepared as described in a previous report [16].

2.2. Cell culture

B16F10 and HaCaT cells were cultured in DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin at 37oC in a 5% CO_2 humidified incubator.

2.3. Cell viability assay

B16F10 cells were seeded at 1×10^4 cells per well in 96-well plates for 24 h and then treated with Pg-C-EE for 48 h. HaCaT cells were seeded at 3.5×10^4 cells per well in 96-well plates for 24 h and then treated with Pg-C-EE for 24 h. Cell viability was measured using the conventional MTT assay. Cells were incubated with 10 μL/well of MTT solution for 3–4 h and then 100 μL MTT stop solution (10% sodium codicil sulfate containing 1M HCl) was added. After 8 h, solubilized formazan was measured by measuring the absorbance at 570 nm using an optical density reader (BioTek, VT, USA).

2.4. Melanin content and secretion analysis

B16F10 cells were seeded at 1×10^5 cells per well in 12-well plates for 24 h and then treated with 100 nM α-MSH, Pg-C-EE, and 1 mM arbutin for 48 h. For the melanin secretion assay, absorbance of culture media was measured using an optical density reader at 475 nm. The cells used for the melanin secretion assay were lysed with 100 mL cell lysis buffer and pelleted by centrifugation (12,000 rpm, 5 min). The pellets were dissolved in 100 mL dissolving buffer (1 M NaOH, 10% dimethyl sulfoxide (DMSO)) and melted at 55°C for 30 min. Absorbance was measured at 405 nm using an optical density reader.

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