Whitening Effects of Angelica dahurica Radix Ethanol Extract in Brown Guinea Pig and Melan-a Cell

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Abstract

The whitening effect of Angelica dahurica Radix ethanol extract (ADEE) was investigated with in vitro and in vivo tests. For in vitro tests, ADEE was treated at concentrations of 6.25, 12.5, 25, and 50 μg/mL to melan-a cells in order to evaluate ADEE’s inhibitory effects on melanin synthesis and tyrosinase activity. Arbutin was used as positive control. For in vivo tests, the test agent was topically applied to hyperpigmented areas on the back skins of brown guinea-pigs (weight: 450 ~ 500 g). The hyperpigmented areas were generated by 1,500 mJ/cm² of ultraviolet B (UVB) irradiation. The test agent of 30 μL was applied twice a day, five days a week, for five weeks. The animals were divided into 6 groups: normal (N), control (C), vehicle control (VC), positive control (PC, 2% hydroquinone), experimental 1 (E1, 1% ADEE) and experimental 2 (E2, 2% ADEE). ADEE effectively inhibited melanin synthesis and tyrosinase activity of melan-a cells in a dose-dependent manner. The pigmentation of brown guinea-pigs was clearly lightened in the PC, E1 and E2 groups compared to the C and VC groups. At the fifth week, the melanin indices of the ADEE groups were significantly lower (p<0.001) than that of the VC group. Visual and quantitative analyses revealed that the degree of melanin pigmentation and S-100 protein expression levels were significantly decreased (p<0.001) in ADEE treated groups compared to control groups.

Keywords: Angelica dahurica Radix, Brown guinea pigs, Melan-a cell, Whitening effect

Introduction

Ultraviolet (UV) radiation is among the most important environmental influences on the human skin. It can induce various skin reactions, including erythema, pigmentation, premature skin aging, and skin cancer (Matsumura & Ananthaswamy, 2004). The skin protects the human body from its environment. Skin is composed of epidermis, dermis, and subcutaneous fat layers (He et al., 2004). Melanin resides in the basal layer of the epidermis and plays an important role in human skin coloring. Melanin is synthesized from melanosomes in the melano-
cytes. Melanosomes contain granular melanin and are transferred from the melanocyte to the keratinocyte nucleus. Here they generate melanin that, inside the keratin, has a turnover of 28 days (Jung et al., 2009). The number of melanocytes does not vary according to race or sex. Differential skin coloring among various races is related to the distribution, number and size of the melanosomes in the keratinocytes. The degree of pigmentation is also decided by heredity, α-melanocyte stimulating hormone levels, and UV exposure (Lin & Fisher, 2007). The tyrosinase activity inside melanin also varies with race, and is higher in black skin types than in Caucasian skin (Fuller et al., 2001). Melanin is an important chromophore in the skin and is able to absorb UV radiation and visible light (Thaler et al., 2009). Although it plays a crucial role in protecting the skin from UV radiation, overproduction and accumulation of melanin in the skin can result in hyperpigmentation disorders (Maeda & Fukuda, 1991).

The melanin pigmentation process in humans comprises three stages. Firstly enzymes needed for melanin synthesis, including tyrosinase, tyrosinase-related protein-1 (TRP1), and dopachrome tautomerase (TRP2), are activated and the function of the melanosome structure is set in motion. The second stage is melanin synthesis, and the third is skin pigmentation by melanin. Melanin concentrations increase through UVB and other external stimuli, as well as through tyrosinase synthesis (Prota, 1980). Tyrosinase is a copper-containing mono-oxygenase acting as a key enzyme in melanin biosynthesis. This enzyme catalyses the oxidation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA), and further to DOPA quinone. The o-quinone is a highly reactive compound and can polymerize spontaneously to form melanin (Land et al., 2003). Therefore, tyrosinase inhibitors have become increasingly important in medicinal and cosmetic products in relation to the treatment of hyperpigmentation (Uyen et al., 2008). Although most of the skin whitening agents currently used (e.g. hydroquinone, kojic acid, arbutin) have high ability to inhibit both melanin production and tyrosinase activity, they present the side-effects of altering melanocyte formation and causing the cells to lose their original activities. And they have been reported to cause irritation and dermatitis under certain conditions (Lin et al., 2007).

It becomes of great interest to search natural products for development of new skin-care cosmetics. Oriental medicine in particular has historically researched such useful plant compounds (Wang et al., 2008). Angelica dahurica Radix (AD), a umbelliferae family member is a medicine with well-known components, including byakangelicol, oxypsoralen, angelic acid, bergapten, xanthotoxin, and scopoletin (Pae et al., 2002), as well as glucids, inorganic materials, and 0.07% essential oils (Joo & Kang, 2005; Kim & Chi, 1990). In vitro research indicates that AD shows antithrombotic, antibacterial, collagen-producing, and antioxidation effects (Lee et al., 2007; Jin et al., 2004; Lechner et al., 2004; Kim et al., 1995). The plant-based AD also contains about 20 kinds of coumarin and essential oil components (Kim et al., 1992). Coumarin is known to have an antithrombotic effect (Kim et al., 1995). The main purpose of this study was to evaluate the potentiality of AD as a whitening product and to establish its whitening mechanisms. The animal studies used brown guinea-pigs, whose skin contains melanocytes and melanosomes similar to those in humans and displays similar reactions to UV rays and chemical products (Yoshida et al., 2002). The whitening effect of Angelica dahurica Radix ethanol extract (ADEE) was verified in the brown guinea-pig skin. And inhibitory effects of ADEE on tyrosinase activity and melanogenesis were evaluated in the melan-a cells.

MATERIALS AND METHODS

1. Materials and apparatus

Hydroquinone (HQ), 3,4-dihydroxy-L-phenyl-alanine (L-DOPA), dimethyl sulfoxide (DMSO), arbutin, L-tyrosine and di (ethylene glycol) reagent were obtained from Sigma Chemical Company (USA). Propylene glycol was purchased from OCI (Korea) and ketamine hydrochloride was obtained from Yu-Han Company (Korea). ADEE (PBC-161A) was obtained from Korea Plant Extract Bank (Korea) and the melan-a cells were obtained from Dr. Dorothy Bennett (St. George’s Hospital, UK). UVB sunlamp (UVM-225D, Mineralight Lamp UVP, USA) was used for UVB irradiation, UV-radiometer (HD 9021, Delta OHM, Italy) was used for UV measuring, and for melanin index measuring mexameter (MX18, CK electronic GmbH, Germany) was used. Inverted microscope (CKX41, Olympus, Japan) was used for observation of the cell line. Fluorescence microscope (Axio imager, Carl Zeiss, Germany) was used for histological observation, and i-solution (IMT i-solution ver. 8.0, Canada) for image analysis.

2. Cell culture

The melan-a cells were grown in Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S) and 200 nM 12-O-etradiacanoylphorbol-13-acetate (TPA) at 37°C,