Inhibitory effect of glyceollin isolated from soybean against melanogenesis in B16 melanoma cells

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INTRODUCTION

Melanin, which is the major pigment of skin, plays an essential role in protection against UV injury under normal physiological conditions (1-3). Melanin biosynthesis is regulated by melanogenic enzymes such as tyrosinase, tyrosinase-related protein-1 (TRP-1) and TRP-2. Tyrosinase is a key enzyme involved in melanin synthesis that can catalyze 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 (5). In the absence of thiols, DOPA quinone changes to DOPA chrome and then to DHI or indole 5, 6-quinone 2-carboxylic acid (DHICA). In addition, there are two further steps in this melanogenic pathway, conversion of DOPA chrome to DHICA, which is catalyzed by TRP-2 (DOPA chrome tautomerase), and the oxidation of DHICA, which is catalyzed by TRP-1 (DHICA oxidase). Microphthalmia-associated transcription factor (MITF) strongly stimulates tyrosinase, TRP-1 and TRP-2, which indicates that it is an important regulator of melanogenesis (6-10).

cAMP increases the expression of MITF through activation of the cAMP-dependent protein kinase A (PKA), which in turn stimulates tyrosinase gene expression to allow melanogenesis and the cAMP pathway is a key physiologic regulator of skin and hair pigmentation in mammals, including humans (12, 13).

Currently, numerous reported pharmacologic and cosmetic agents inhibit melanin biosynthesis targets. Many skin lightening products such as linoic acid, hinokitol, kojic acid, naturally occurring hydroquinone and catechol have been reported to inhibit melanogenesis (14); however, these compounds exhibited side effects, toxicity and low clinical activity. These adverse effects have led to the search for compounds that lack side effects, such as natural molecules derived from plant extract based skin lightening products (15). Therefore, many plants have been investigated to determine their potential for use as cosmetic agents.

Glyceollin is one of a group of phytoalexins produced in soybeans under stress conditions (16, 17). Glyceollin (mixture of glyceollin I, II, and III), which has been produced in high concentrations using several elicitors, exerts antimicrobial activity against several plant pathogens (18, 19). Existing reports previously identified glyceollin as anti-estrogenic agents that may be useful in the prevention or treatment of prostate, breast and ovarian carcinoma (20, 21). However, the effects of glyceollin on melanogenesis have not been evaluated to date. In the present study, we evaluated the inhibitory activity of glyceollin against melanin biosynthesis and its mechanism of action in B16 melanoma cells.
RESULTS

Effects of glyceollin on the cytotoxicity of B16 melanoma cells
Pure glyceollin (mixture of glyceollin I, II, and III) was isolated from elicited soybean using the procedure developed by Boue et al. (22). To determine if pure glyceollin has cytotoxic effects, we treated B16 melanoma cells with glyceollin at various concentrations. The cell viability was then determined by an MTT assay. As shown in Fig. 1, glyceollin does not exert a cytotoxic effect against B16 melanoma cells at concentrations ranging from 0.1-50 μM.

Inhibitory effects of glyceollin against α-MSH enhanced melanogenesis and tyrosinase activity
Melanocytes can be stimulated by many effectors, including ultraviolet radiation (UV) and α-MSH. In this study, we used α-MSH (10 nM) to stimulate melanogenesis in B16 melanoma cells. As shown in Fig. 2A, the extracellular melanin contents of cells decreased significantly in the glyceollin range (0.1-10 μM). Moreover, intracellular melanin reduction by glyceollin was observed (Fig. 2B). These results indicate that glyceollin inhibits melanin synthesis in B16 melanoma cells.

Tyrosinase is a key enzyme involved in melanin biosynthesis. Therefore, the inhibition of tyrosinase is a major strategy for development of new whitening agents. The effects of glyceollin on the catalytic activities of tyrosinase are shown in Fig. 2C. The pure glyceollin has inhibitory effects against the oxidation activity of mushroom tyrosinase in a dose-dependent fashion. Furthermore, in the cell-based tyrosinase assay, inhibition of the tyrosinase activity by glyceollin was found to occur in a dose-dependent manner in B16 melanoma cells (Fig. 2D). It should be noted that the reduced melanin contents were attributed to the suppression of tyrosinase activity in a cell-free system and under cellular conditions.