PD 4) Suppression of alpha-MSH and IBMX induced melanogenesis by cordycepin via inhibiting CREB and MITF, and activation of PI3K/Akt and ERK-dependent mechanisms

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1. Introduction

Melanin, which is synthesized in intracellular organelles designated melanosomes, is a major pigment of skin color. Melanogenesis is regulated by three specific enzymes: tyrosinase, tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2). Tyrosinase, a copper-containing glycoprotein, is a key enzyme in melanin synthesis and a rate-limiting enzyme in this pathway, and can catalyze three different reactions: the hydroxylation of tyrosine to 3, 4-dihydroxyphenylalanine (DOPA), the oxidation of DOPA to DOPA-quinone changes to DOPA-chrome, and then to dihydro-indolizine (DHI) or indole 5,6-quinone2-carboxylic acid (DHICA)(1-3). During this biosynthesis pathway, TRP-1 and TRP-2 have functional roles. TRP-1 catalyzes the oxidation of DHICA, and TRP-2 (DOPA chrome tautomerase) catalyzes the conversion of DOPA-chrome to DHICA(4). In addition, the two enzymes are regulated by a specific transcription factor, microphthalmia-associated transcription factor (MITF)(5-7). Cordycepin is extracted from fungi of genus Cordyceps,(has been used traditionally as an anti-tumor, anti-viral, anti-inflammatory agent in East Asian countries) (8-11). In this study, we first to show that the inhibitory effect of cordycepin on the melanogenesis signaling pathways, including the expression of MITF, tyrosinase, TRP1 and TRP2, and the phosphorylation of CREB, via the activation of ERK and Akt in murine B16F10 melanoma cells stimulated by a-MSH and IBMX.

2. Materials and methods

Materials:Cordycepin, a-MSH, IBMX, L-3, 4-dihydroxyphenylalanine (L-DOPA), arbutin, and
Cell culture. Cells of the B16-F10 murine melanoma cell line obtained from the American Type Culture Collection (Rockville, MD, USA) were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL). The cells were incubated at 37°C in a humidified atmosphere composed of 5% CO₂ and 95% air. To avoid changes in cell characteristics produced by extended cell culture periods, cells were used between passages 15 and 25. Each cell suspension was split every 2 days to maintain exponential growth. Cells were treated with 1 µM α-MSH in the absence or presence of cordycepin for 48 h. The cell pellets were then dissolved in 500 µl of 1 N NaOH in 10% DMSO at 80°C for 1 h. Melanin production was calculated as described above.

3. Results and Discussion

Cordycepin inhibits α-MSH- and IBMX-induced cellular melanin synthesis and tyrosinase activity. Prior to investigating the pharmacological potential of cordycepin on α-MSH- and IBMX-induced melanogenesis activity, the dose and time dependence of the cytotoxic effects of 0.5–20 mM cordycepin in B16F10 cells were assessed using the MTT viability assay. The different concentrations of cordycepin that were used were not cytotoxic, the results were shown as Fig. 1D. The effect of cordycepin on α-MSH- and IBMX-stimulated melanin synthesis is summarized in Fig1. A and B. α-MSH and IBMX are crucial cAMP-elevating agents, they act differently. α-MSH combines the melanocortin 1 receptor (MC1R) and activates adenylate cyclase, which can raise the intracellular cAMP concentration. IBMX increases the intracellular cAMP concentration by inhibiting cAMP phosphodiesterase. Presently, 5–20 µM cordycepin inhibited melanin synthesis induced by both α-MSH and IBMX (Fig1. A and B). The efficacious concentrations of cordycepin are much lower than the effective level (200µM) of arbutin, an established effective melanogenesis inhibitor and popular cosmetic agent. Cordycepin also decreased the melanin synthesis relative protein tyrosinase activity (Fig. 1C).

References