Antimelanogenic Activity of 3,4-Dihydroxyacetophenone: Inhibition of Tyrosinase and MITF

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3,4-Dihydroxyacetophenone (3,4-DHAP) was evaluated for antimelanogenic activity. The tyrosinase inhibitory action by 3,4-DHAP using mushroom tyrosinase revealed a strong inhibitory effect. To further explore this matter, inhibition of tyrosinase and melanin content was measured in B16 melanoma cells (B16 cells). Further, tyrosinase and microphthalmia transcription factor (MITF) protein levels were determined by the Western blot method. Additionally, tyrosinase and MITF protein levels were reduced by 3,4-DHAP. Our data indicate that the antimelanogenic activity of 3,4-DHAP was probably due to its inhibition of tyrosinase activity and the suppression of tyrosinase and MITF protein levels.

Key words: antimelanogenic activity; 3,4-dihydroxyacetophenone; melanin; microphthalmia transcription factor; tyrosinase

The control of melanogenesis is an important strategy in the treatment of abnormal skin pigmentation for cosmetic purposes.1) Modulators of melanogenesis can act directly on tyrosinase, tyrosinase gene expression, or the mechanisms responsible for the transfer of melanosomes from melanocytes to keratinocytes.2) It has been reported that transcription factors such as lymphoid-enhancing factor 1 are involved in the expression of tyrosinase-related protein.3) Recently, another transcription factor, microphthalmia transcription factor (MITF), was shown to play a key role in melanocyte survival, development and differentiation.2) In recent papers, we reported that 4,4'-dihydroxybiphenyl has a strong tyrosinase inhibitory effect that down-regulates melanogenesis,9) and that protocatechuic aldehyde has a potent tyrosinase inhibitory effect.5)

The present work was designed to investigate the mode of inhibitory action of 3,4-dihydroxyacetophenone (3,4-DHAP) on mushroom tyrosinase activity and on tyrosinase activity and melanin content in B16 melanoma cells. Further, to gain molecular insight into the inhibition of melanogenesis by 3,4-DHAP, we investigated its effect on tyrosinase and MITF protein molecules. 3,4-DHAP is known as a vasoactive agent and antioxidant,6,7) but its inhibitory effect on melanogenesis and the modulation of MITF is first reported, in the present study.

In this study, the inhibitory action of 3,4-DHAP on tyrosinase was assayed as described previously.8) Twenty µl mushroom tyrosinase (1,000 units) was added to a 96-well microplate, which was incubated at 25 °C for 30 min. The amount of dopachrome produced in the reaction mixture was determined spectrophotometrically at 492 nm. We calculated individual IC50 when Y-axis showed 50% of the inhibition percentage.

As Fig. 1 indicates, 3,4-DHAP had a strong suppressive action against tyrosinase activity, with a IC50 value of 10 µM. To establish the relative efficacy of 3,4-DHAP, its inhibitory effects were compared with a well-known tyrosinase inhibitor, kojic acid. The inhibitory effect of 3,4-DHAP was significantly stronger (IC50 = 1.0 × 10−7 m) than that of kojic acid (IC50 = 2.6 × 10−7 m).

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Note

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melanocyte-stimulating hormone (α-MSH). In our work, we investigated the inhibitory effect of 3,4-DHAP on the tyrosinase activity of B16 cells treated with α-MSH. B16 cells were cultured in DMEM with 10% fetal bovine serum and penicillin/streptomycin (100 IU/50 µg/ml) in a humidified atmosphere containing 5% CO₂ in air at 37°C. B16 cells were incubated in the presence of 100 nM α-MSH and then treated for 24 h with 3,4-DHAP. The cells were lysed in 100 µl of 50 mM sodium phosphate buffer (pH 6.8) containing 1% Triton X-100 (Sigma, Kyoto, Japan) and 0.1 mM phenylmethylsulfonyl fluoride, and centrifuged at 12,000 rpm for 30 min at 4°C. The supernatant and 3,4-dihydroxyphenylalanin (2 mg/ml) were placed in a 96-well plate, and the absorbance at 492 nm was read using an ELISA plate reader. As shown in Fig. 2A, 3,4-DHAP effectively inhibited tyrosinase activity as compared to the α-MSH-treated group.

We also examined melanogenesis in B16 cells. Determination of melanin content was performed using a modification of the method of Bilodeau et al.9 Briefly, B16 cells (5 × 10⁵) were then incubated for 24 h with or without 3,4-DHAP. The cells were then incubated for 24 h with or without 3,4-DHAP. After they were washed twice with phosphate buffered saline, samples were dissolved in 100 µl of 1 N NaOH. Absorbance at 405 nm was compared with a standard melanin. The results showed that melanin synthesis was effectively inhibited in a dose-related manner (Fig. 2B).

In support of the mechanism by which 3,4-DHAP reduces melanin synthesis in cultured B16 cells, we determined tyrosinase and MITF protein levels by the Western blot method, as described below. Western blotting detection reagents were obtained from Amersham (Bucks, UK), RNAzolTM B was obtained from Tel-Test, Inc. (Friendswood, Texas, USA). Polyclonal antibody to MITF was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The rabbit polyclonal anti-tyrosinase (pep7) antibody was from Dr. V. Hearing (Bethesda, Maryland). Monoclonal sheep anti-mouse IgG antibody and donkey anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody were purchased from Serotec (Oxford, UK). The B16 cells (5 × 10⁵) were incubated in multi-dishes with 100 nM α-MSH for 24 h with or without 3,4-DHAP. The cells were lysed in buffer (40 mM Tris, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40, 0.1 mM sodium orthovanadate, 2 µg/ml aprotinin, 2 µg/ml leupeptin and 100 µg/ml phenylmethylsulfonyl fluoride), and the supernatant was collected. For Western blotting, equal amounts of proteins were boiled for 2 min and chilled on ice, subjected to 8–10% SDS gel electrophoresis, and transferred to a polyvinylidene difluoride membrane. The proteins were visualized with the enhanced chemiluminescence detection system.

As Fig. 3 shows, 3,4-DHAP suppressed the amount of tyrosinase protein.

Our data are correlate well with previous reports that the induction of melanogenesis in B16 cells is characterized by the stimulation of tyrosinase activity and tyrosinase gene expression.11) In the present study, we documented that 3,4-DHAP suppresses melanogenesis and tyrosinase activity by modulating the protein amounts of tyrosinase and MITF.
In this regard, a recent report\textsuperscript{12} on the inhibition of melanin and MITF by epigallocatechin-3-gallate and hinokitiol is worthy of note.

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**References**


