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ORIGINAL ARTICLE

Inhibition of tyrosinase activity and melanine pigmentation by 2-hydroxytyrosol

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KEY WORDS

2-Hydroxytyrosol;
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Melanine formation;
B16 melanoma cells

Abstract 2-Hydroxytyrosol (2-HT), originally reported as a synthetic compound, was isolated for the first time as a fungal metabolite. 2-HT was found to inhibit mushroom tyrosinase with an IC_{50} value of 13.0 μmol/L. Furthermore, 2-HT dose-dependently inhibited tyrosinase activity (IC_{50}, 32.5 μmol/L) in the cell-free extract of B16 melanoma cells and α-melanocyte stimulating hormone (α-MSH)-stimulated melanin formation in intact B16 melanoma cells.

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1. Introduction

Melanin is essential for protecting human skin against radiation, but the accumulation of abnormal melanin induces pigmentation disorders, such as melasma, freckles, ephelides, and senile lentigines[1]. Melanogenesis is conducted in melanocytes, located in the basal layer of the epidermis and controlled by tyrosinase[2].

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase enzyme involved in melanogenesis[3]. The enzyme is widely distributed in fungi, higher plants and animals[4], and is involved in the first two steps of the melanin biosynthesis, in which L-tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (L-DOPA, monophenolase activity) and the latter is subsequently oxidated to dopaquinone (diphenolase activity)[2]. A large number of moderate to potent tyrosinase inhibitors from natural and synthetic resources have been reported during the last decade[5–9]. Tyrosinase inhibitors such as arbutin, kojic acid and hydroquinones have been used as whitening or antihyperpigment agents because of their ability to suppress dermal-melanin production[10,11]. However, arbutin and kojic acid hardly showed inhibitory activity against pigmentation in intact melanocytes or in a clinical trial[12], and hydroquinones are considered to be cytotoxic to melanocytes and potentially mutagenic to mammalian cells[11]. Therefore, it remains necessary to search for new tyrosinase inhibitors without side effects.

During our course of screening for mushroom tyrosinase inhibitors of microbial origin, 2-hydroxytyrosol (2-HT, Fig. 1) was isolated from the fungal culture broth of *Metarhizium* sp. OB-0098. 2-HT was originally reported to be a synthetic compound[13], but its biological activity has not been reported. In this study, tyrosinase inhibitory activities and melanin formation in mouse B16 melanoma cells of 2-HT were described.

2. Results

2.1. Inhibition of mushroom tyrosinase activity by 2-hydroxytyrosol

In this assay, the conversion of L-DOPA to dopaquinone by mushroom tyrosinase was observed at 450 nm. As shown in Fig. 2, 2-HT dose-dependently inhibited mushroom tyrosinase activity with an IC_{50} value of 13.0 μmol/L. Under the same conditions, kojic acid also inhibited the activity with IC_{50} of 14.8 μmol/L.

2.2. Inhibition of melanin pigmentation in B16 melanoma cells by 2-hydroxytyrosol

To investigate whether 2-HT inhibited melanogenesis, the effect of 2-HT on melanin pigmentation in intact B16 melanoma cells was studied. α-MSH was added to this assay system, because melanin production was markedly enhanced. 2-HT was found to inhibit the melanin pigmentation of B16 melanoma cells in a dose-dependent manner with IC_{50} of 571 μmol/L (Fig. 3). Under the same conditions, arbutin inhibited the melanin pigmentation with IC_{50} of 1130 μmol/L, and kojic acid inhibited it by 45.7% at 735 μmol/L. Furthermore, the cytotoxic effects of these inhibitors on B16 melanoma cells were investigated by the MTT assay. The IC_{50} values of 2-HT, kojic acid and arbutin were 1.3, 3.0 and 1.8 mmol/L, respectively.

2.3. Inhibition of B16 cells tyrosinase activity by 2-hydroxytyrosol

To confirm the inhibition of melanin pigmentation in intact B16 melanoma cells by 2-HT, the effect of 2-HT on tyrosinase activity

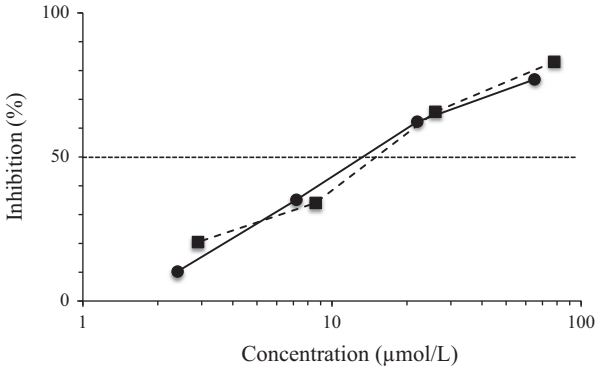

Figure 2 Inhibitory effects of 2-HT(●) and kojic acid (■) against mushroom tyrosinase.

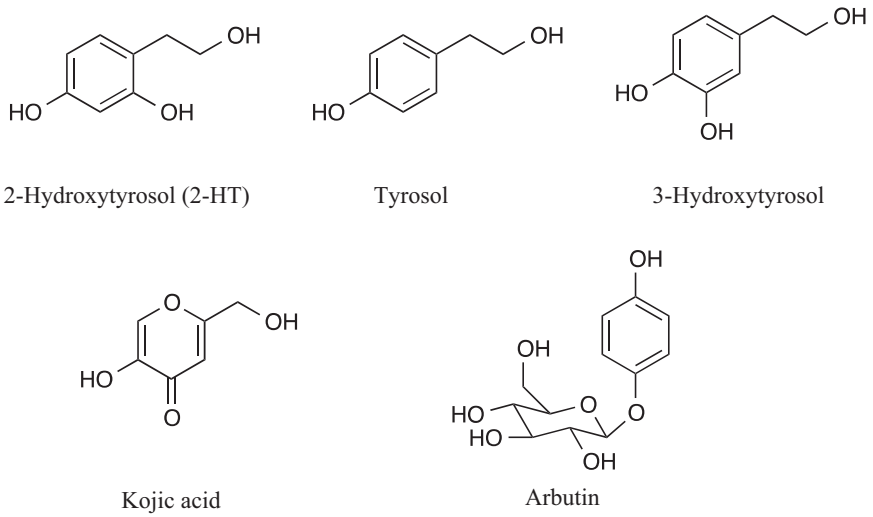

Figure 1 Structures of 2-hydroxytyrosol (2-HT) and tyrosinase inhibitors.