

Assessment of the Effect of Arbutin Isomers and Kojic Acid on Melanin Production, Tyrosinase Activity, and Tyrosinase Expression in B16-4A5 and HMV-II Melanoma Cells

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Abstract

The inhibitory effects of α -arbutin, β -arbutin, and kojic acid on melanogenesis, tyrosinase activity, and tyrosinase protein expression in mouse melanoma cells (B16-4A5) and human melanoma cells (HMV-II) were directly compared. β -Arbutin showed a stronger inhibitory effect on melanogenesis and tyrosinase expression in B16-4A5 cells than α -arbutin and kojic acid. Kojic acid showed a stronger inhibitory effect on mushroom and B16-4A5 tyrosinase activity than α -arbutin and β -arbutin. In contrast, kojic acid inhibited all of these effects more strongly than α -arbutin or β -arbutin in HMV-II cells. These results suggest that kojic acid may be used as a positive control for the inhibitory melanogenesis assay, and for tyrosinase activity and tyrosinase expression assays that use HMV-II cells. Moreover, using HMV-II cells with kojic acid as the positive control may facilitate the search for new skin-whitening agents using natural products and provide an alternative to the B16-4A5 assay.

Key words

α -arbutin · β -arbutin · kojic acid · melanin · tyrosinase

Supporting information available online at <http://www.thieme-connect.de/products>

The production of excessive pigmentation in melasma, spots, freckles, and other forms of hyperpigmentation can pose a significant aesthetic problem [1,2]. The causative pigment, melanin, is formed by a combination of enzymatically catalyzed chemical reactions. Melanogenesis is initiated by the catalytic oxidation of tyrosine to dopaquinone by tyrosinase; this is the rate-limiting step in melanin synthesis [3]. Tyrosinase inhibitors reduce or block melanin synthesis, leading to skin whitening. Several common skin-lightening and depigmentation agents available commercially include arbutin (hydroquinone β -D-glucopyranoside, β -ARB), kojic acid (KA), and hydroquinone [4,5]. These compounds are isolated from natural resources and are bioassayed using mushroom tyrosinase and B16 mouse melanoma cells. In a recent study, human melanoma HMV-II cells were used as an alternative to B16 [6,7]. α -Arbutin (α -ARB), the epimer of β -ARB (Fig. 1), has inhibitory effects on melanogenesis and tyrosinase [8,9]. However, it remains unclear whether β -ARB or α -ARB has strong activity against HMV-II, and the inhibitory effects of KA on melanogenesis and tyrosinase using HMV-II are also unclear.

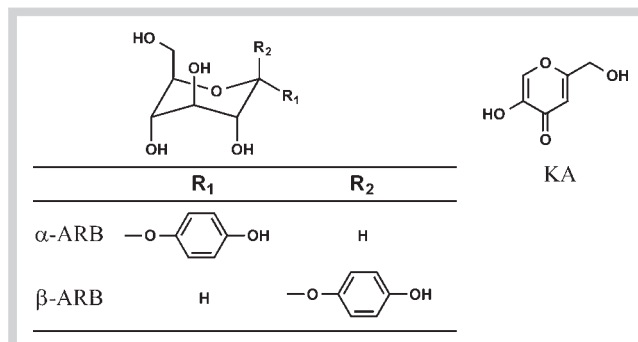


Fig. 1 Structures of α -ARB, β -ARB, and KA.

In this study, we directly compared the inhibitory effects of these compounds on melanogenesis and tyrosinase activity using B16 and HMV-II.

The inhibitory effect of α -ARB, β -ARB, and KA on the proliferation and pigmentation of B16-4A5 and HMV-II were compared. Fig. 2A shows that α -ARB, β -ARB, and KA at 125 to 1000 μ M suppressed melanogenesis in B16-4A5. β -ARB significantly suppressed melanogenesis in a concentration-dependent manner between 125 and 1000 μ M, α -ARB significantly suppressed melanogenesis in a concentration-dependent manner between 250 and 1000 μ M, whereas KA only significantly suppressed melanogenesis at 1000 μ M. These results showed that β -ARB suppressed melanogenesis more effectively than α -ARB and KA in B16-4A5.

Fig. 2B shows that α -ARB, β -ARB, and KA at 125 to 1000 μ M suppressed melanogenesis in HMV-II, KA significantly suppressed melanogenesis in a concentration-dependent manner between 125 and 1000 μ M, whereas α -ARB and β -ARB significantly suppressed melanogenesis in a concentration-dependent manner between 500 and 1000 μ M. These results demonstrated that KA was superior compared to α -ARB and β -ARB at suppressing melanogenesis in HMV-II. The inhibitory effects of these compounds on melanogenesis in HMV-II were, thus, in contrast with their inhibitory effects on melanogenesis in B16-4A5. Significant cytotoxic effects were only observed for α -ARB at 1000 μ M on B16-4A5, β -ARB at 500 and 1000 μ M on B16-4A5, and β -ARB at 1000 μ M on HMV-II (Fig. 1 S, Supporting Information).

The inhibition of tyrosinase or/and downregulation of tyrosinase expression are mechanisms by which depigmentation occurs [3, 10]. Mushroom tyrosinase, B16-4A5, and HMV-II are used to screen for inhibitors of tyrosinase and suppressors of melanin biosynthesis [3, 4, 11]. Table 1 shows the inhibitory effect of α -ARB, β -ARB, and KA on mushroom, B16-4A5, and HMV-II tyrosinase. α -ARB interestingly showed an inhibitory effect on B16-4A5 tyrosinase ($297.4 \pm 9.7 \mu$ M) but no inhibitory effect on mushroom and HMV-II tyrosinases ($> 500 \mu$ M). In contrast, β -ARB showed weak inhibition of all three tyrosinases ($> 500 \mu$ M). The KA IC_{50} inhibition values of mushroom, B16-4A5, and HMV-II tyrosinases were $297.4 \pm 9.7 \mu$ M, $57.8 \pm 1.8 \mu$ M, and $223.8 \pm 4.9 \mu$ M, respectively.

We postulated that the melanogenesis inhibitory effect of KA might have contributed to the inhibition of tyrosinase. To investigate the mechanism by which α -ARB, β -ARB, and KA inhibit melanogenesis, we used Western blotting to examine the effect of these compounds on the expression of B16-4A5 and HMV-II tyrosinase proteins (Fig. 3). In B16-4A5, tyrosinase expression was decreased by β -ARB, α -ARB, and KA (all 500 μ M, listed in order of

decreasing effect) and this ordering of the compounds is the same as the order of their effect on reducing melanin in B16-4A5. In HMV-II, tyrosinase expression was decreased by KA, β -ARB, and α -ARB (all 1000 μ M, listed in order of decreasing effect),

which is the same order as their effect on reducing melanin content. KA is a known tyrosinase inhibitor, but it does not decrease tyrosinase expression in several B16 cell lines [12]. However, there are no previous reports directly comparing the effect of KA with other agents in decreasing tyrosine levels in HMV-II, as apposed to B16 tyrosinase levels. The present data confirmed that the inhibition of melanogenesis by KA is due to a specific inhibition and downregulation of HMV-II tyrosinase.

In conclusion, KA directly inhibited tyrosinase activity and suppressed the expression levels of tyrosinase in HMV-II. Furthermore, this is the first report to directly compare the effects of α -ARB, β -ARB, and KA on melanogenesis and to evaluate their inhibitory effect on tyrosinase in B16-4A5 and HMV-II. These results suggest that KA can be used as a positive control in the assessment of melanogenesis, tyrosinase activity, and tyrosinase expression in HMV-II cells, thereby providing an alternative to the mushroom tyrosinase assay and B16-4A5 assay in the search for new skin-whitening agents derived from natural products (Table 2).

Materials and Methods



Cell cultures: B16-4A5 (RCB0557) and HMV-II (RCB0777) cells were obtained from the RIKEN BRC through the National Bio-Resource Project of the MEXT, Tsukuba, Japan. B16-4A5 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Nichirei Biosciences), penicillin, and streptomycin at 37 °C in a humidified atmosphere consisting of 5% CO₂ and 95% air.

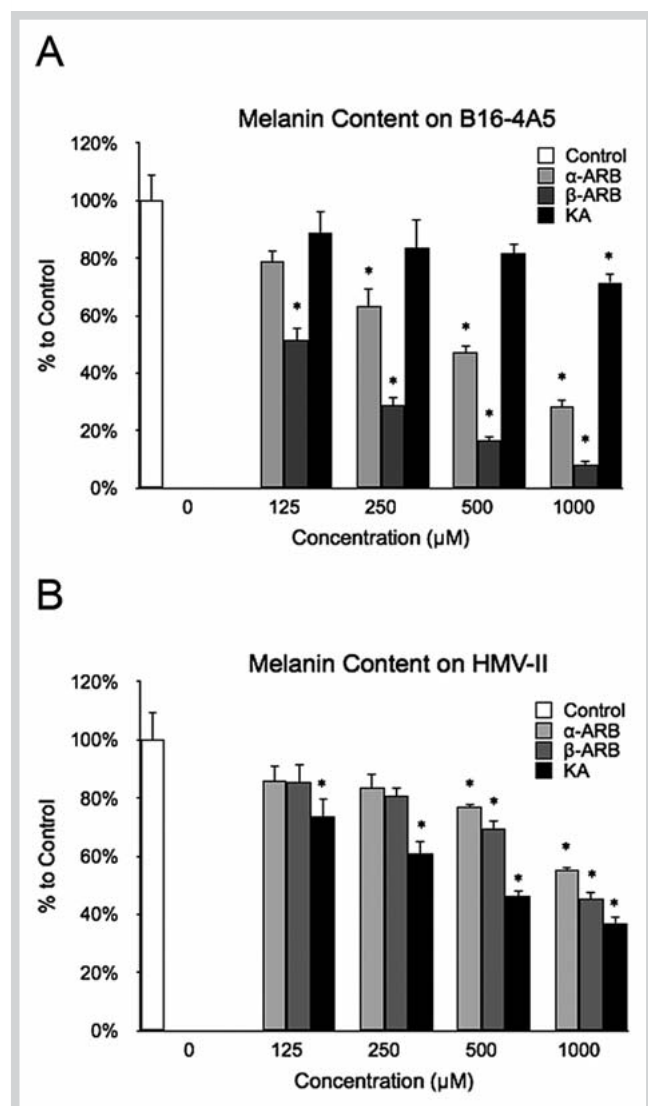


Fig. 2 Inhibitory effect of α -ARB, β -ARB, and KA on melanogenesis in B16-4A5 (A) and HMV-II (B) cells. Each compound at 125, 250, 500, or 1000 μ M was individually added to the cells on the first and fourth day of cell culture. On day 7, the number of viable cells in each culture was determined using 4% Alamar Blue reagent. Each column represents the mean \pm SD of four independent tests (Student's t-test). * $P < 0.01$, significantly different from the control value.

Table 1 IC₅₀ values (μ M) of the tyrosinase activity inhibitory effect of α -ARB, β -ARB, and KA.

	Mushroom tyrosinase	B16-4A5 tyrosinase	HMV-II tyrosinase
α -ARB	> 500	297.4 \pm 9.7	> 500
β -ARB	> 500	> 500	> 500
KA	182.7 \pm 9.8	57.8 \pm 1.8	223.8 \pm 4.9

Values for each compound represent the mean \pm SD of four independent tests

Table 2 Potential candidates for bioassays for new skin-whitening agents extracted from natural products.

	Mushroom	B16-4A5	HMV-II
Melanin production	–	β -ARB	KA
Tyrosinase activity	KA	KA	KA
Tyrosinase expression	–	β -ARB	KA

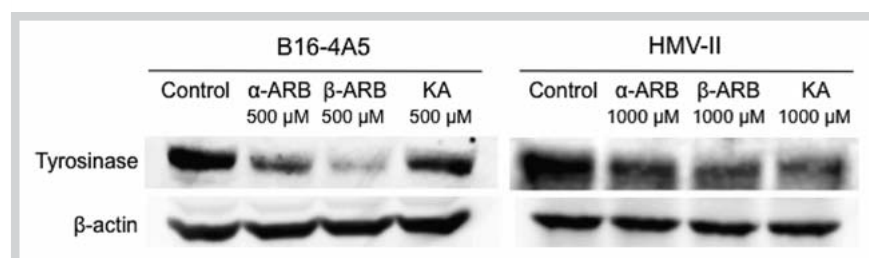


Fig. 3 Inhibitory effect of α -ARB, β -ARB, and KA on melanin production in B16-4A5 and HMV-II cells by downregulation of tyrosinase expression. The cells were treated with the compounds for 72 h. The expression levels of tyrosinase were examined by Western blot using antibodies.

HMV-II cells were cultured in Ham's F12 supplemented with 10% FBS (Vitromex), penicillin, and streptomycin at 37 °C in a humidified atmosphere consisting of 5% CO₂ and 95% air.

Measurement of melanin content: The amount of melanin in cultured melanoma cells was measured as described previously [13, 14]. See Supporting Information for detailed protocols.

Tyrosinase inhibition assay: Mushroom tyrosinase activity was measured by determining its DOPA-oxidase activity using a modification of the method of Ha et al. [15]. First, 120 μL of L-DOPA (8 mM, dissolved in 50 mM phosphate buffer, pH 6.8) and 40 μL of the sample were mixed. Then, 40 μL of mushroom tyrosinase (80 units/mL) was added, and the amount of dopachrome in the reaction mixture was determined by measuring the optical density at 492 nm after 20 min at 37 °C.

Expression of tyrosinase in B16-4A5 and HMV-II cells: Crude tyrosinase was prepared based on the method of Ohguchi et al. [16]. Tyrosinase expression was confirmed by Western blotting [17]. See Supporting Information for detailed protocols.

Supporting information

Detailed information on materials and methods are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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