Effects of Kojic acid, Arbutin and Vitamin C on cell viability and melanin synthesis in B16BL6 cells

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Synopsis

Research objective: To exactly evaluate their functions of kojic acid, arbutin and vitamin C as a whitening agent, we performed experiments to compare their abilities to inhibit melanin synthesis.

Experimental methods and techniques: The effects of kojic acid, arbutin and vitamin C on cell viability and melanin synthesis were evaluated by the level of melanin content and the number of viable cells upon treatment of them.

Main observations: According to in vitro tyrosinase inhibition assay, we found that while kojic acid and hydroquinone have strong inhibition activities(their IC_{50}s are all <100uM), arbutin and vitamin C have weak inhibitory activities. In B16BL6 melanoma cells, like in vitro tyrosinase inhibition assay, arbutin and kojic acid showed more strong inhibition effect on melanin synthesis than vitamin C. And we also found the fact that both vitamin C and kojic acid induce cell death at high concentration, whereas arbutin induces al change of cellular morphology at high concentration

instead of cell death..

Major conclusions: .. In this paper, we suggest both kojic acid and arbutin have stronger ability to inhibit melanogenesis than vitamin C. And they also have side effect, that is, while both kojic acid and vitamin C induces cell death, arbutin changes cell morphology .

Key words: Melanogenesis, Cell viability, Kojic acid, Vitamin C, Arbutin

Introduction

Melanin biosynthesis is a human defense mechanism to protect skin from UV irradiation and also determines colors of hair and skin[1]. However, as a interest on skin-whitening increases, researches which are designed to prevent hypersynthesis of melanin in skin are being actively in progress[2]. Active components used as a whitening agent in cosmeceuticals are kojic acid, arbutin, vitamin C and hydroquinone[3, 4, 5, 7]. However, until now, comparitive studies about them in the aspect of both melanin formation and cellular toxicity have not been performed, Because of this, we can't exactly estimate merits and defects of them as a whitening agent. To this end, we performed experiments to compare their effects on cell viability and melanin formation.

Experiments

.Cell Cultures- B-16/BL6 murine melanoma cells were cultured in Minimum essential medium(MEM) with 10% fetal bovineserum and penicillin/streptomycin(100IU/50 g/ml) in a humidified atmosphere containing 5% CO_2 in air 37°C.

Melanin content assay-Melanin contents of cultured B16/BL6 cells were measured according to the method of Oka M. et al. (1996) with a slight modification[8]. The colors of cell pellets were evaluated visually, and pellet were solubilized in boiling 1M NaOH for 10min. Spectrophotometric analysis of melanin content was performed at 400nm absorbance.

MTT assay-General viability of cultured cells was determined by reduction of 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan[9, 10, 11, 12].

After GR treatment, cells incubated for 24h at 37C in 5% CO2 atmosphere. MTT (1mg/ ml in PBS) was added to each well, 1/10 volume of media. Cells were incubated at 37C for 3h, and dimethyl sulfoxide was added to dissolve the formazan crystals. The absobance was then measured at 570nm with a spectrophotometer.

Assay of tyrosinase activity-Cell were washed twice with PBS and lysed. Cell lysates of melanoma cells were prepared using Triton X-100 and subjected to Tyrosinase activity assay. Substrate solution [0.1% L-DOPA in 0.1M sodium phosphate(pH 6.0)] 100 ul and 50 ul of enzyme solution was incubated 37C. The initial rate of linear increase in

absorbance at 475nm, based on the formation of dopachrome, was measured using a spectrpphotometer.

Result and discussion.

To exactly characterize properties of arbutin, kojic acid and vitamin C, as a first step, *in vitro* tyrosinase inhibition assay was performed. As we expected, while kojic acid showed strong inhibition activities(their IC_{50}s are all <100uM), indicating the same effect as hydroquinine used as a positive control, arbutin and vitamin C showed weak activities. IC_{50}s of arbutin and vitamin C are 100uM and 400~500uM, respectively(Fig. 1). These results indicate that all four chemicals act as a a inhibitor of tyrosinase.

As a further study, we performed melanin content assay to know that they also play the same function in mammalian cells. Through this melanin content assay in B16BL6 melanoma cells, we found that arbutin and kojic acid have more strong inhibitory effect on melanin synthesis than vitamin C, consistent with result in *in vitro* tyrosinase activity assay(Fig. 2). And unlike arbutin, vitamin C and kojic acid induced cell death at high concentration(Fig. 3). Although arbutin showed no cytotoxicity, it has side effect to induce morphological change at high concentration(Fig. 4). In this paper, we suggest both kojic acid and arbutin have stronger ability to inhibit melanogenesis than vitamin C. And they also have side effect,respectively, that is, while both kojic acid and vitamin C induce cell death, arbutin changes cell morphology in B16BL6.

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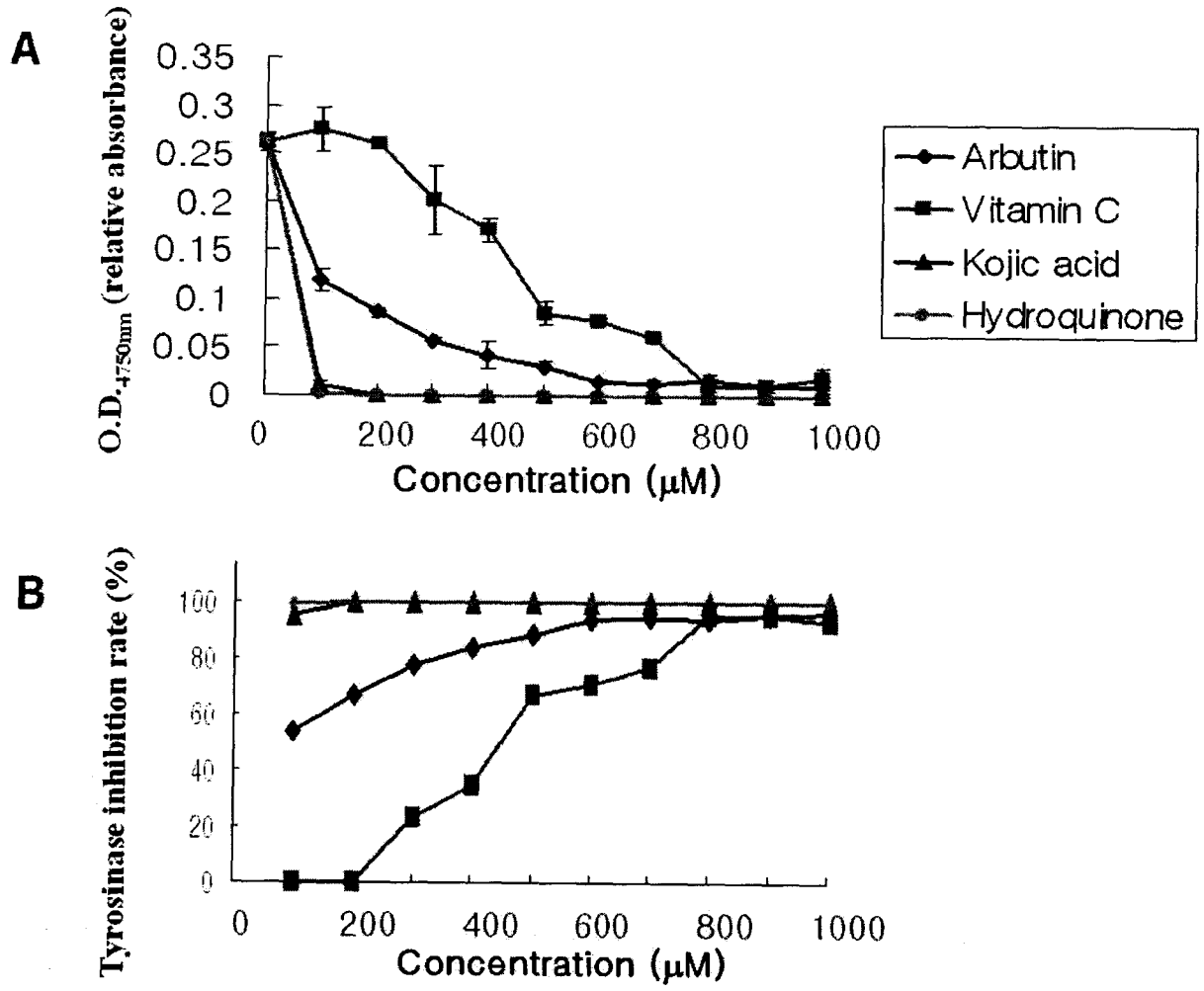

Fig. 1 In vitro inhibition test of tyrosinase activity.

L-tyrosine is converted into L-dopa by tyrosinase. Concentration of L-dopa can be determined by spectrophotometry. This experiment shows that whitening agents such as Vitamin C, Kojic acid and arbutin inhibit tyrosinase activity in a dose-dependent manner. IC_{50} of whitening agents is as follows; 100mM(Arbutin), 400mM~500mM(Vitamin C), <100mM(Kojic acid and Hydroquinone)

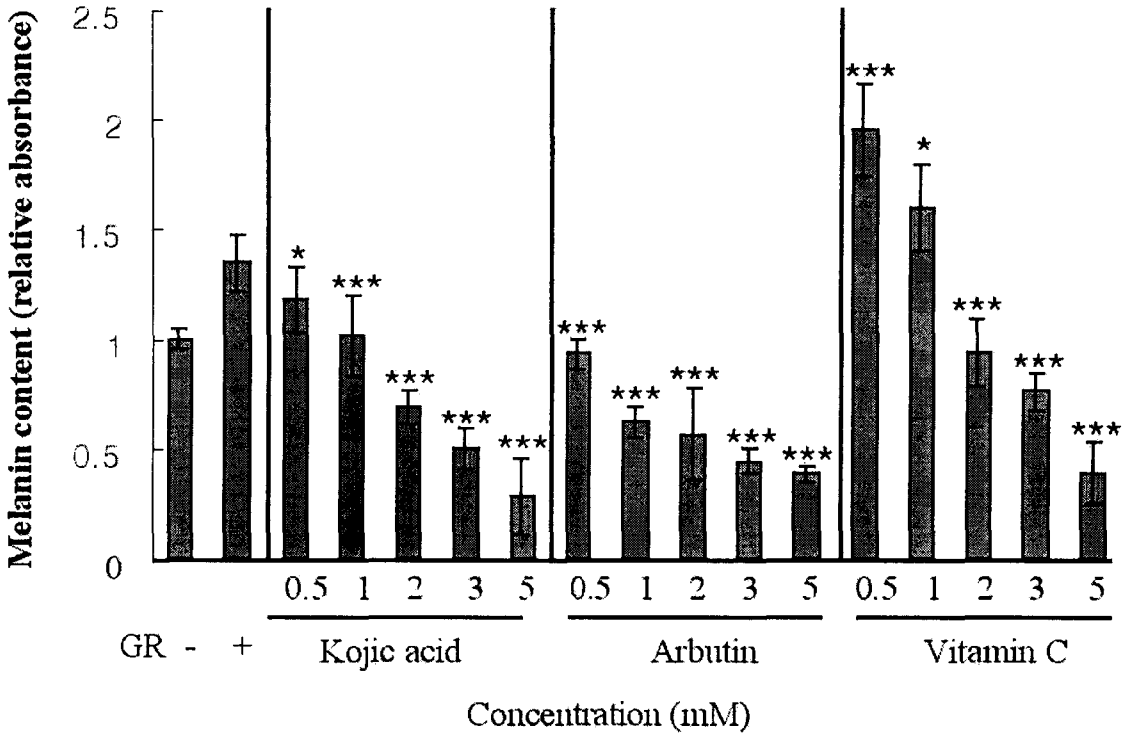

Fig. 2 Melanin content assay was done according to the method, previously described. Result are expressed as relative absorbance of control and data means ±SD of at least three determinations. (* P<0.1, ** P<0.01, ***P<0.001, n=9)

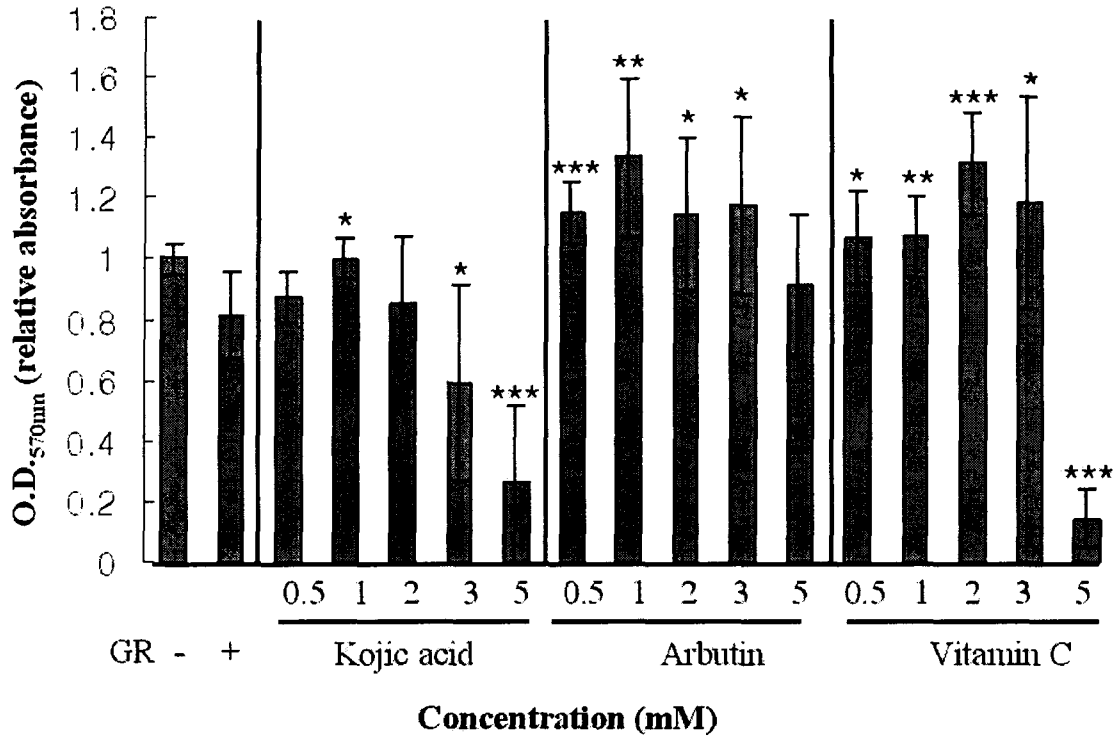

Fig.3. Effect of whitening agents on the cell. Cell viability was determined by measuring optical density(OD) at 570nm (* P<0.1, ** P<0.01, ***P<0.001, n=9)

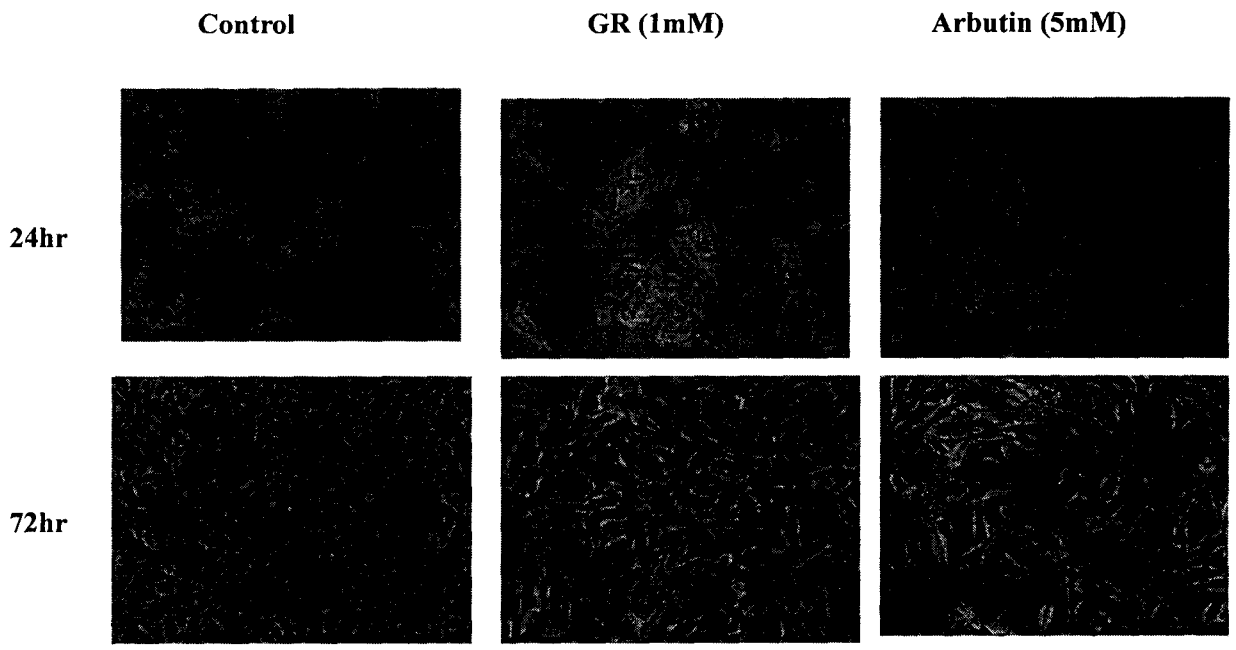

Fig. 4 Cell morphology of B16BL6 melanoma cell, treated without or with GR and arbutin 5mM, respectively for 24hrs or 72hrs.(x100)