Anti-melanogenesis effect of unripe *Rubus occidentalis* L. extract and its active component, ellagic acid in B16F10 mouse melanoma cells

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B16F10 mouse melanoma 세포에서 미숙과 복분자 추출물과 
유효성분인 ellagic acid에 의한 멜라닌 생성의 억제 효과

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요 액

*Rubus occidentalis* L. is a deciduous broadleaf shrub that belongs to family *Rosaceae*. It has been shown to have various effects including anti-cancer and anti-inflammatory. In addition, unripe *Rubus occidentalis* L. is reported to have higher polyphenol content than ripe one. In this study, we aimed to investigate the effect of unripe *Rubus occidentalis* L. (uRo) and its active component ellagic acid (EA) on anti-melanogenesis. uRo extract and EA inhibited mushroom tyrosinase activity in a dose-dependent manner. We also found that uRo and EA markedly inhibited melanin production and tyrosinase activity in B16F10 melanoma cells. In addition, uRo and EA decreased the expression of melanogenesis-related proteins such as tyrosinase-related protein-1 (TRP-1) and tyrosinase-related protein-2 (TRP-2). These results indicate that uRo extracts and EA contributes to reduce melanin synthesis via inhibition of tyrosinase activity and down-regulation melanogenesis-related proteins. Therefore, uRo extract and EA has the potential to be used as functional cosmeceutical ingredients.

1. Introduction

The synthesis of melanin is synthesized from melanosome within the cytoplasm of melanocytes and regulated by ultra violet light, cytokine, growth factor and hormone, and through various mechanisms within the cell [1]. Tyrosinase is an enzyme that catalyzes in the oxidation process of L-tyrosine, being hydroxylated into L-dihydroxyphenylalanine (L-DOPA) and L-DOPA being oxidized to dopaquinone and finally plays an important role in producing melanin polymer polymers [2]. In addition, TRP-1 and TRP-2 are important factors involved in melanin biosynthesis [3]. Whitening agents such as arbutin, kojic acid, L-ascorbic acid are known to inhibit melanin synthesis by reducing tyrosinase activity. However, these compounds have side effects such as excesses melanin degradation and stability problems [4]. Therefore, it is necessary to develop a new therapeutic agents for skin pigmentation. *Rubus occidentalis* L. has been used as one of the traditional Korean medicine for treatment of prostatism, diabetes mellitus, impotence, spermatorrhea, enuresis, and asthma [5]. It has also been reported that anti-cancer and anti-inflammatory effects are also excellent. Studies on various physiologically active substances and functionalities of *Rubus occidentalis* L. fruit have been actively carried out with increasing interest in its efficacy. The kinds of physiological active substances present in the fruit include sanguiin H-4, gallic acid, quercetin, ellagic acid and various phenolic acids [6]. In particular, ellagic acid is a polyphenol compound in various fruits, and its research results indicate that it exhibits anticancer and antioxidant activity [7]. Therefore, we investigated and compared the anti-melanogenesis effect of uRo and EA, to confirm their possibility as functional cosmetics ingredients.

2. Material & Methods

Mushroom tyrosinase activity assay was 160 mL of 5mM L-DOPA (in 100mM sodium phosphate buffer pH 6.8) and
20 mL of the same buffer with and without the test sample were placed in the wells of a 96-well microplate, and then 20 mL of mushroom tyrosinase (200 units/mL) were mixed into each well at 37 °C over 30 min. Cell viability was measured by MTT assay. In B16F10 cells, uRo (0.3-300 μg/mL) extract and EA (0.03-30 μM) was treated for 48 h. MTT solution was added to each well, and the cells were incubated at 37 °C for 3 h, and dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. The melanin content was determined by solubilization of the cell pellet in 1 N NaOH in 10% DMSO at 80 °C for 1 h. Intracellular tyrosinase activity was measured by adding 50 μL of 0.1% L-DOPA at 37 °C for 1 h by measuring the ratio of L-DOPA. The Western blot analysis was conducted by separating cell lysates by SDS-gel electrophoresis. The gels were transferred on NC membranes, and exposed to the appropriate antibodies. Statistical analysis of the experimental data points was performed using an ANOVA and Duncan's multiple range test using SPSS 22.0 (IBM, SPSS, Chicago IL, USA) software. A value of p<0.05 was considered to be statistically significant.

3. Result and Discussion

We evaluated the inhibitory effects of uRo extract and EA on mushroom tyrosinase. uRo extract and EA were found to inhibit mushroom tyrosinase activity at concentrations of 300 μg/mL and 30 μM, respectively (Fig. 1). As shown in Fig. 2, uRo extract and EA did not show cytotoxic effects on B16F10 cells in the concentration range 0.3-100 μg/mL and 0.03-10 μM, respectively. Thus doses without cytotoxicity were chosen to determine the effects of uRo extract and EA on tyrosinase activity and melanin synthesis. To verify the ability of uRo extract and EA on α-MSH-mediated melanogenesis, we determined the quantity of intracellular melanin and tyrosinase activity in the presence of α-MSH. As shown in Fig. 3, uRo extract and EA substantially decreased the α-MSH-induced cellular melanin contents in a dose-dependent manner. Likewise, uRo extract and EA also reduced the α-MSH-induced intracellular tyrosinase activity in a dose-dependent manner (Fig. 4). These results suggest that uRo extract and EA may reduce melanin synthesis by inhibiting tyrosinase activity in B16F10 cells. To elucidate the mechanisms underlying the anti-melanogenesis activities of uRo extract and EA, we first examined the effects of uRo extract and EA on the expression levels of the melanogenic enzymes using Western blot analysis. As shown in Fig. 5, uRo extract and EA decreased expression of melanogenesis-related proteins such as TRP-1 and TRP-2. In conclusion, we have demonstrated that uRo extracts and EA decrease melanin synthesis via inhibition of tyrosinase activity and down-regulation of melanogenesis-related proteins. Therefore, uRo extract and EA may be a useful therapeutic agent or an effective cosmetic ingredient for improving hyperpigmentation.
[Fig. 3] Inhibitory effect of uRo and EA on melanin synthesis on α-MSH treated B16F10 cells. The B16F10 cells were incubated with α-MSH (100 nM), uRo (10–100 μg/mL) extracts, EA (1–10 μM) and Ar (100 μg/mL) for 48 h. Result are means±SD of three independent experiments. Significance was determined using ANOVA. **p<0.01, ***p<0.001. α-MSH-treated control: ##p<0.01. (Nor: normal-control, Con: control, uRo: unripe Rubus occidentalis L., EA: Ellagic acid, Ar: Arbutin)

[Fig. 4] Inhibitory effect of uRo and EA on intracellular tyrosinase activity on α-MSH treated B16F10 cells. The B16F10 cells were incubated with α-MSH (100 nM), uRo (10–100 μg/mL) extracts, EA (1–10 μM) and Ar (100 μg/mL) for 48 h. Result are means±SD of three independent experiments. Significance was determined using ANOVA. *p<0.05, **p<0.01, ***p<0.001. α-MSH-treated control: ###p<0.01. (Nor: normal-control, Con: control, uRo: unripe Rubus occidentalis L., EA: Ellagic acid, Ar: Arbutin)

[Fig. 5] Effect of uRo extract and EA on TRP−1 and TRP−2 expression in α-MSH stimulated B16F10 melanoma cells. Cells were exposed to α-MSH (100 nM) alone or plus with uRo extract (100 μg/mL) and EA (100 μg/mL) or arbutin (100 μg/mL) for 48 h. GAPDH was used as an internal control.

참고문헌


