

Effect of simultaneous administration of vitamin C, L-cysteine and vitamin E on the melanogenesis

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Abstract. The effect of simultaneous administration of vitamin C (ascorbic acid), L-cystein (Cys) and vitamin E (tocopherol) on the melanogenesis *in vivo* and *in vitro* was studied.

Forty-eight brownish guinea pigs were divided into 4 groups as follows: VC group, VC+Cys group, VC+Cys+VE group and control group. They were given these vitamins by oral administration every day. UV-B exposure (0.384 J/cm²) on their depleted back skin was done at the day 8, 10, 12, 15 17 and 19. After UV-B irradiation, vitamins were administrated further 3 weeks. The luminosity score was measured using a Color Reader CR-11 (Minolta, Co) and the numbers of DOPA-positive melanocytes of their back skin were counted. B16 melanoma cells were incubated with VC, N-acetyl cystein (NAC) and VE. After 4 days of incubation, cells were harvested. The melanin contents and the tyrosinase activities in cells were measured.

The luminosity score in the VC+VE+Cys group was higher than those in the other groups. The numbers of DOPA-positive melanocytes of guinea pigs treated with VC, VE and Cys were significantly decreased compared with those in VC group. In B16 melanoma cells, simultaneous treatment of VC, VE and NAC was the most effective to decrease the melanin contents and to inhibit tyrosinase activity.

Keywords: Melanin, tyrosinase, ascorbic acid, tocopherol, cystein, UV irradiation

1. Introduction

Vitamin C (ascorbic acid) is well known to inhibit melanogenesis. The various derivatives of ascorbic acid have developed to permeate via surface of skin for the purpose of the use as application agent [1]. As the problem of damage by UV irradiation is getting serious, protection by oral supplementation is also expected. Oral supplementation of only ascorbic acid is not so effective that we investigated the effect of simultaneous administration of vitamin C (ascorbic acid, AsA), L-cystein (Cys) and vitamin E (tocopherol, α -Toc) on the melanogenesis *in vivo* and *in vitro*.

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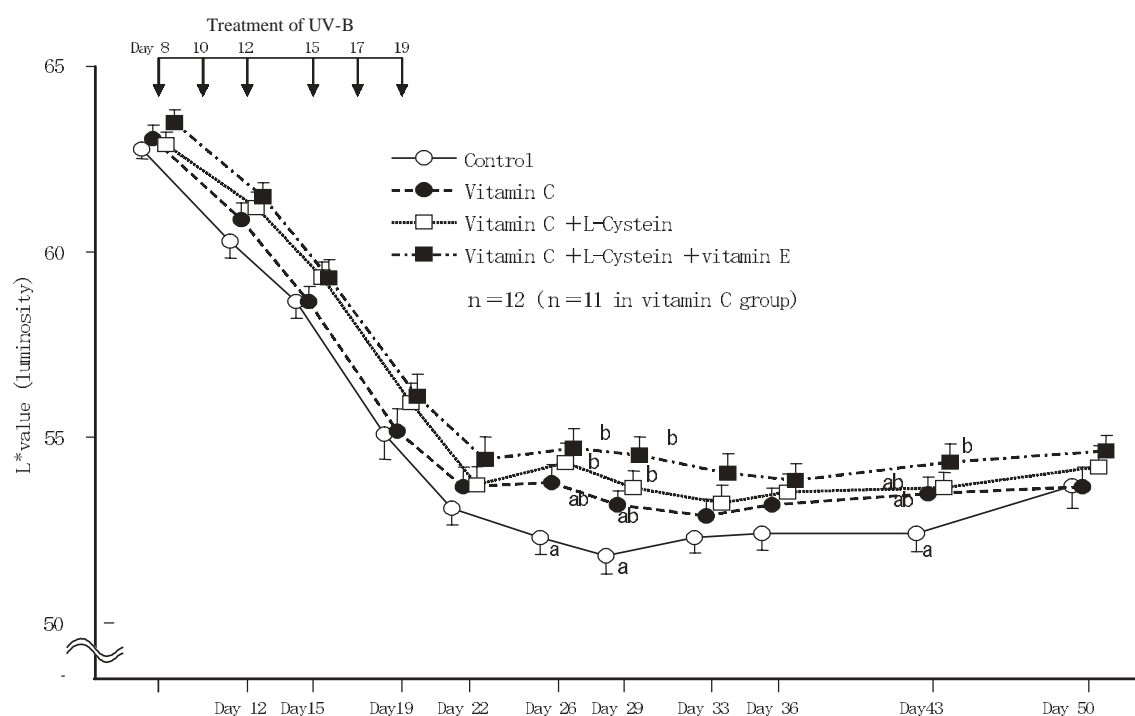


Fig. 1. Changes of L* scores during experiments. Guinea pigs were given vitamins by oral administration every day during the experiment. UV-B exposure (0.384 J/cm^2) on their depleted back skin was done at the day 8, 10, 12, 15 17 and 19. The luminosity score (L* score) was measured using a Color Reader CR-11 (Minolta, Co). Values with the different superscript show significant differences in the same day by Tukey's test. Mean \pm SE ($n = 12$).

2. Oral administration of simultaneous AsA, Cys and α -Toc inhibited melanogenesis in guinea pigs exposed by UV irradiation

Forty-eight brownish guinea pigs were divided into 4 groups as follows: VC group (AsA 600 mg/kg), VC+Cys group (AsA 600 mg/kg + Cys 160 mg/kg), VC+Cys+VE group (AsA 600 mg/kg, + Cys 160 mg/kg + α -Toc 50 mg/kg) and control group (no treatment). They were given by oral administration every day. UV-B exposure (0.384 J/cm^2) on their depleted back skin was done at the day 8, 10, 12, 15 17 and 19. After UV-B irradiation, vitamins were administrated further 3 weeks. The luminosity score (L* score) was measured using a Color Reader CR-11 (Minolta, Co) and the numbers of DOPA-positive melanocytes of their back skin were counted. L* scores were getting down until the day 22, after UV-B exposure (Fig. 1). Figure 1 showed that administration of vitamins protected the decrease in L* scores. The L* score in the VC+VE+Cys group was higher than those in the other groups, indicating that simultaneous treatment was more effective than the only AsA treatment to inhibit the accumulation of pigmentation by UV irradiation. The numbers of DOPA-positive melanocytes of guinea pigs treated with VC, VE and Cys were also significantly decreased compared with those in VC group (data not shown). TBARS of guinea pigs after UV irradiation was the lowest in VC+VE+Cys group, suggesting that VE would trap the peroxyradical caused by UV-B. Pigmentation is caused by various chemical mediators and cytokines, such as endothelin [2], pro-opiomelanocortin [3], induced by UV-B irradiation. Although the relationship between the oxidative stress and the secretion of these cytokines is not known, VE may act as a radical scavenger and inhibit the production of melanogenic cytokines.

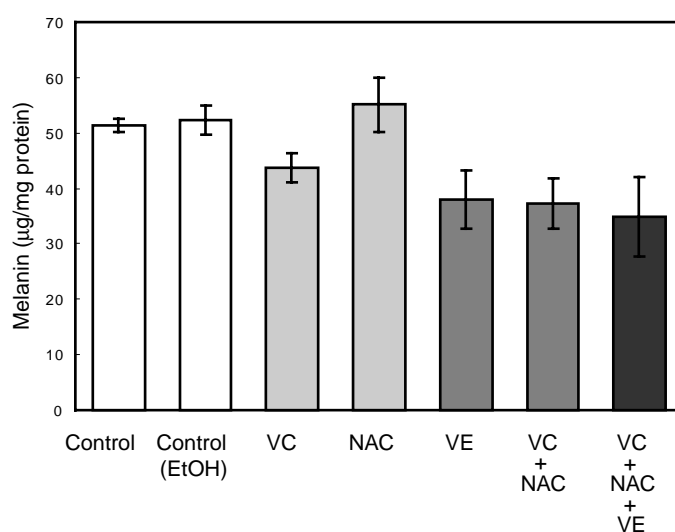


Fig. 2. Melanin concentrations of B16 melanoma cells treated with various vitamins. B16 melanoma cells were incubated with AsA (25 µg/ml), NAC (80 µg/ml), α -Toc (50 µg/ml) and their combination. After 4 days incubation, melanin concentrations in melanoma were measured. Mean \pm SD ($n = 3$).

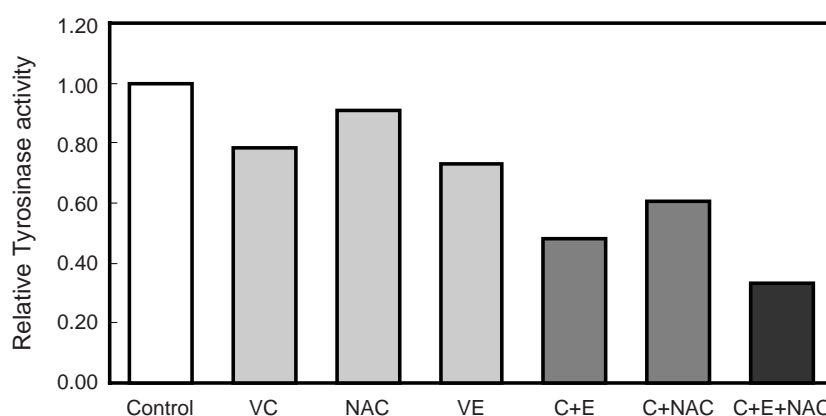


Fig. 3. Relative inhibition of tyrosinase activity in melanoma cells. B16 melanoma cells were incubated with AsA (25 µg/ml), NAC (80 µg/ml), α -Toc (50 µg/ml) and their combination. After 4 days incubation, cells were harvested. Their homogenates were used for kinetic study using the DOPA as the reaction substrates. Values were indicated with the average of triple experiments.

3. Combination of vitamin treatment decreased the melanin contents and tyrosinase activity in B16 melanoma

B16 melanoma cells were incubated with AsA (10 µg/ml), N-acetyl cystein (NAC, 80 µg/ml) and α -Toc (50 µg/ml). After 4 days of incubation, cells were harvested. The melanin contents were measured as their absorbance at 470 nm after hydrolysis by NaOH at 80°C. Tyrosinase activity was investigated with kinetic study of the reaction of DOPA using the cell homogenates. AsA and α -Toc but not NAC significantly reduced the concentrations of melanin in B16 melanoma cells. However, addition of NAC to ASA decreased more and simultaneous AsA, NAC and α -Toc was the most effective to reduce the

melanin (Fig. 2). Cys is known to lead DOPA into cystenyl-DOPA, resulting in the produce of brownish pheomelanin but not the dark melanin [4]. Since we were not able to distinguish these two types of melanins with the method we used here, additional effect of NAC is though to be synergic. As shown in Fig. 3, inhibition of tyrosinase activity was the strongest in the simultaneous treatment of AsA, NAC and α -Toc.

In conclusion, oral administrations of AsA indicated the efficacy to inhibit melanogenesis by further addition of simultaneous Cys and α -Toc. One of the mechanisms is suggested to be their synergic effect as the radical scavenger against oxidative stress caused by UV irradiation. Synergistic effect of simultaneous treatment as shown in B16 melanoma might cause the protective effect by UV exposure.

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