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# Superior Skin Protection via Astaxanthin

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It has been believed for a long time that the skin exists only for the purpose of merely protecting our body by physically shielding it from outside factors. But in recent years, along with the radical progress in the field of dermatological science studies, it is known that the skin does actually indicate various responses and accept acute and chronic damages under UV irradiation. According to the enthusiastic studies to clarify the mechanism leading to the skin damages, nowadays the reactive oxygen species generated by UV irradiation is considered to be an important factor mediating photo-induced skin damages. Accumulated skin damages by reactive oxygen species such as lipid peroxidation, sunburn and degenerative changes in dermal connective tissues induce the skin aging. To protect skin from reactive oxygen species, many cosmetics contain nowadays both naturally occurring molecules and synthetic compounds as antioxidant. However,  $\beta$ -carotene was the only carotenoid for cosmetics among more than 600 carotenoids which had isolated from nature, until astaxanthin from antarctic krill was approved for cosmetics in 1997. In this paper, I would like to show the possibility of astaxanthin as a cosmetic ingredient and the useful formula for maintaining the stability of astaxanthin in the preparation.

## 1. Singlet oxygen ( $^1\Delta_g$ ) scavenging activity of astaxanthin

Compared with other nonspecific techniques for the detection of singlet oxygen, direct observation of the singlet oxygen emission at 1268 nm has proven to be the most reliable approach for singlet oxygen detection [1,2]. We have constructed a sensitive near-infrared emission spectrometer with a germanium (Ge) detector which can scan from 1000 to 1600 nm [3-5]. We applied this apparatus for the comparison of singlet oxygen scavenging activity of various cosmetic ingredients. Singlet oxygen scavenging activity was evaluated by the rate constant for quenching singlet oxygen from Stern-Volmer prot.

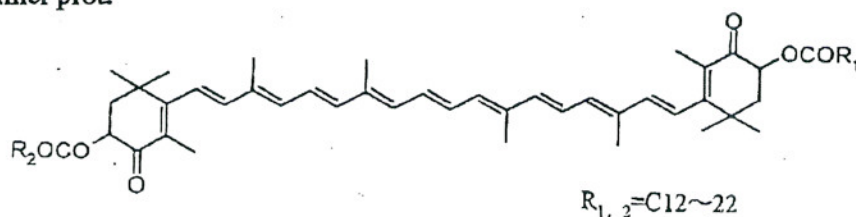


Fig.1 Astaxanthin

The singlet oxygen scavenging activity of astaxanthin (Fig.1) was found to be much greater than that of cosmetic ingredients used for antioxidant such as  $\beta$ -carotene,  $\alpha$ -tocopherol, rutin, phospholipid and thiotaurine. It is known that singlet oxygen is actually generated on the skin surface from *Propionibacterium acnes* porphyrin under UV irradiation [6]. These results suggest that topically applied astaxanthin, which scavenges singlet oxygen effectively, can play an important role to protect the skin from various photodamages such as lipid peroxidation, sunburn reaction, phototoxicity and photoallergy induced by singlet oxygen.

## 2. Melanogenesis inhibition by astaxanthin in mouse melanoma cells

Skin pigmentation, such as suntan, stains and freckles, is caused by excessive uneven production of melanin which is synthesized by melanocytes due to such factors as UV rays. It is known that pigmentation in the skin is prevented by reducing tyrosinase activity, either by inhibiting the synthesis of tyrosinase, which is an important enzyme for melanin synthesis, or by using an antagonist of the substrate for tyrosinase. Pigmentation is also prevented by inhibiting auto-oxidation of dopa and suppressing inflammatory reactions, such as erythema, that occur following UV irradiation. Various whitening agents which are known to inhibit tyrosinase activity have been used to treat the pigmentation include arbutin, kojic acid and ascorbic acid derivatives.

We investigated the inhibitory effect of astaxanthin on melanogenesis. B16 mouse melanoma cells were cultured with astaxanthin for three days, and the tyrosinase activity was assayed by using L-DOPA as a substrate. In addition, cell viability of three-day cultures was evaluated. Astaxanthin dose-dependently reduced tyrosinase activity at final concentrations between 2.5  $\mu$ M and 10  $\mu$ M, at which no change in cell viability was seen (Fig.2). The amount of melanin was reduced to 40% by 10  $\mu$ M astaxanthin. This action was stronger than that of arbutin, kojic acid and ascorbic acid derivatives. Astaxanthin did not inactivated isolated tyrosinase, indicating that it may prevent melanin synthesis by inhibiting the auto-oxidation of dopa and dopaquinone.

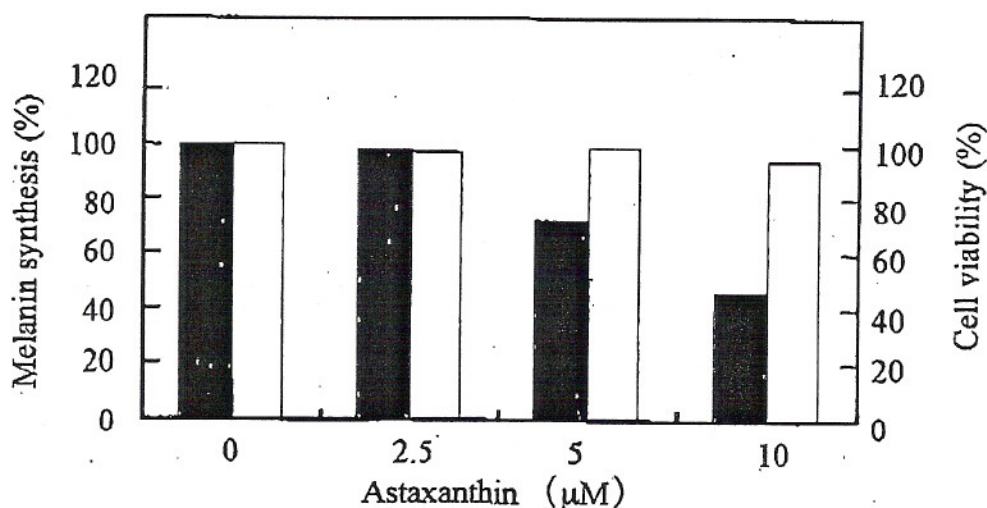


Fig.2 Melanogenesis inhibition by astaxanthin

### 3. Photoprotective effect of topically applied astaxanthin to skin

The wrinkles which appear in the skin are for many one of the most frustrating aspects of aging because they are symbolic of the inevitable decline which occurs with the passage of time. Experimental studies conducted on animals, as well as examinations of human skin chronically exposed to solar irradiation, have confirmed that photoaging is clearly one of the most important causes of wrinkle formation[7-9].

It is known that reactive oxygen species is deeply involved in the UV-induced photoaging. Information from in vitro research suggests that it is singlet oxygen generated by UV irradiation that is very important in degradation of collagen which induces the degeneration of ultrastructure of dermal collagen fiber bundles and the decrease in skin elasticity and the wrinkle formation[10-11]. For these reasons, we focused on the application of astaxanthin as a singlet oxygen scavenger to inhibit the UV-induced photoaging.

Using hairless mice irradiated with UVB to produce photoaged skin, the effect of astaxanthin on appearance of wrinkles, skin elasticity and ultrastructural changes of dermal collagen fiber bundles were examined. Mice were irradiated five times weekly with 65-95 mJ / cm<sup>2</sup> of UVB irradiation for 18 weeks. The back skin of mice was treated with 0.1 ml of vehicle or astaxanthin (350 µM) in vehicle. These topical treatments were done after UVB irradiation. We evaluated wrinkle formation using a 6-grade scoring system and the wrinkle score of the mouse skin irradiated with UVB for 18 weeks is shown in Fig. 3. In the group exposed to UVB irradiation, wrinkling was significantly increased compared with age-matched non-irradiated controls. On the other hand, the wrinkle score of the group treated by astaxanthin after UVB irradiation was lower than that of vehicle treatment. Scanning electron micrographs of the ultrastructure of dermal collagen fiber bundles indicated that the application of astaxanthin yielded remarkable maintenance of the bundle structures, accompanied by a reduction in wrinkles, that is essential in keeping health and function of skin.

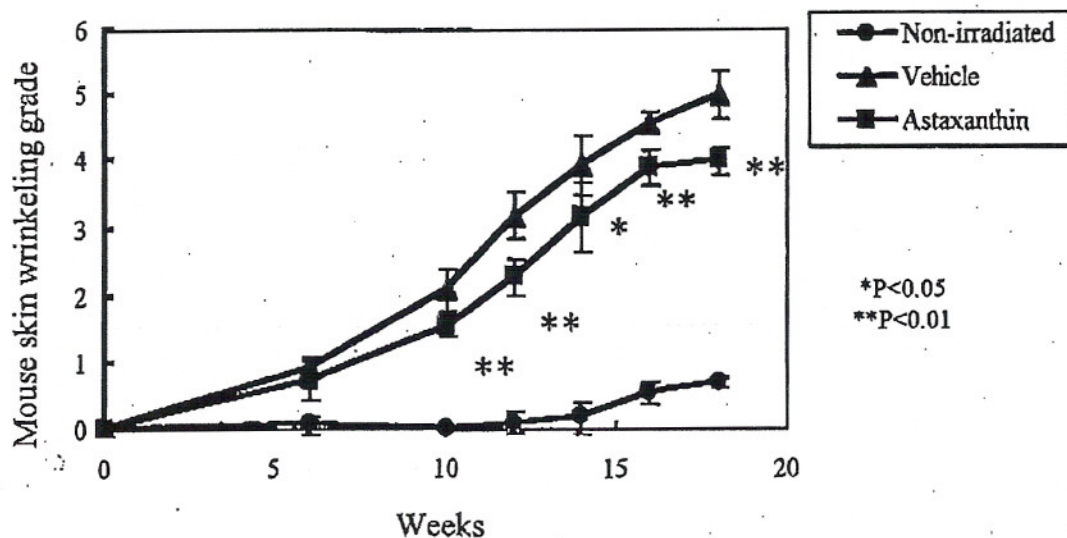


Fig.3 Photoprotective effect of topically applied astaxanthin to skin

In summary, astaxanthin showed a superior protection effect on photoaging in hairless mice. We were convinced that astaxanthin would be a novel and promising antioxidant for cosmetic applications to prevent chronic photoaging.

#### 4. Useful formula to maintain long-term stability of astaxanthin in preparation

It is well known that carotenoids are easy to decompose during storage by UV light and oxygen. Many applications of carotenoids for cosmetics have been tried over the years, but the instability of carotenoids has made it difficult for successful results. In order to utilize astaxanthin in cosmetics, the preservation of astaxanthin for wide-range thermal conditions under UV light and oxygen is necessary.

We investigated the factors affecting the stability of astaxanthin during storage under UV light without bubbling nitrogen gas into the preparation. The kind of surfactant was very important to stabilize astaxanthin. The incorporation of  $\alpha$ -tocopherol and  $\alpha$ -glucosyl rutin each prevented decomposition of astaxanthin. We found that the ratio of  $\alpha$ -tocopherol and  $\alpha$ -glucosyl rutin were very important to stabilize astaxanthin in the preparation. When we used  $\alpha$ -tocopherol and  $\alpha$ -glucosyl rutin in the ratio 1:100, the decomposition of astaxanthin was not observed. Our formula can maintain astaxanthin over a period of 6 months even under UV light without bubbling nitrogen gas.

#### 5. References

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