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# Protective Effect of Fucoxanthin against UVB-Induced Skin Photoaging in Hairless Mice

Itaru URIKURA, Tatsuya SUGAWARA,† and Takashi HIRATA

*Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*

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**Fucoxanthin, a major carotenoid in brown algae, has various beneficial effects. In this study, we evaluated the effect of topical fucoxanthin on UVB-induced skin photoaging in hairless mice. The dorsal skins were treated topically with a 0.001% fucoxanthin solution 2 h each time before UVB irradiation (5 times a week) for 10 weeks. The formation of wrinkles in UVB-irradiated skin treated with vehicle alone significantly increased, as compared with the non-irradiated control. Treatment with fucoxanthin tended to suppress UVB-induced wrinkle formation, but there was no significant difference between wrinkle formation in the control group and the fucoxanthin treatment group. However, topical treatment with fucoxanthin significantly lessened UVB-induced epidermal hypertrophy, VEGF, and MMP-13 expression in the epidermis and thiobarbituric acid reactive substances (TBARS) in the skin. These results indicate that topical treatment with fucoxanthin prevents skin photoaging in UVB-irradiated hairless mice, possibly *via* antioxidant and antiangiogenic effects.**

**Key words:** fucoxanthin; photoaging; wrinkling; UV-irradiation; angiogenesis

Photoaging caused by repeated exposure to sunlight involves the premature aging of skin, clinically characterized by dryness, pigmentation, laxity, and wrinkling.[1] In particular, short-wavelength UV causes photodamage, including erythema, wrinkling, and skin cancer. UV-induced generation of reactive oxygen species (ROS) can result in structural and functional alterations in the extracellular matrix (ECM), *e.g.*, collagen, elastin, and glycosaminoglycans, which can contribute to photoaging. ROS also cause direct deleterious chemical modifications to cellular components, including DNA, proteins, and lipids.[2,3]

In addition, exposure to UV radiation leads to inflammatory reactions in the skin. Neutrophils are the first infiltrating cells to migrate into UV-irradiated skin. Besides the ECM damage directly induced by UV-induced ROS, neutrophil-derived proteolytic enzymes, including matrix metalloproteinases (MMPs), are also important contributors to ECM damage during skin photoaging.[4] Furthermore, it has been found that UVB-induced angiogenesis of the skin is associated with a switch in the balance of vascular endothelial growth factor (VEGF) and thrombospondin (TSP)-1 expression, which also plays an important role in skin photodamage.[5,6] VEGF promotes sensitivity to UVB-induced skin photodamage and wrinkling.[7]

Fucoxanthin is a major carotenoid of edible brown seaweeds, such as *Undaria pinnatifida*, *Hijikia fusiformis*, and *Sargassum fulvellum* (Fig. 1). Recently, there have been several reports showing that fucoxanthin has beneficial effects on human health, as in cancer prevention and anti-obesity.[8–13] We hypothesized that fucoxanthin contributes to prevention of the skin photoaging induced by UV, since it has anti-oxidative,[14–16] anti-inflammatory,[17–19] and anti-angiogenic effects,[20] but there are no previous reports on the effects of fucoxanthin on chronic UV-induced skin photodamage. In this study, we examined the effect of fucoxanthin on UVB-induced photoaging in the skin of hairless mice by evaluating various parameters of photoaging.

## Materials and Methods

*Materials.* Fucoxanthin was extracted and purified from brown alga (*Undaria pinnatifida*), as reported previously.[21] Briefly, an acetone extract of the brown algae was applied to a silica gel (Keisel gel 60, Merck, Darmstadt, Germany) column, and was eluted by stepwise elution with an hexane:ethyl acetate mixture (10:0–4:6, v/v). Fucoxanthin was recovered in the hexane:ethyl acetate fraction (5:5–4:6, v/v). The fucoxanthin-rich fraction was further subjected to flash-column chromatography on a LiChroprep RP-18 (40–63 μm, 11 × 240 mm, Merck) with acetonitrile/methanol/water (75:15:10) containing 0.1% ammonium acetate to isolate pure fucoxanthin.

*Animals.* Six-week-old female HOS:HR-1 hairless mice (Clea Japan, Tokyo) were housed at 24 ± 1 °C under a 12-h light-dark cycle. They were fed standard chow (MF, Oriental Yeast, Tokyo) and water *ad libitum*, and were allowed to acclimate for 2 weeks before the start of the experiments. They were randomly divided into 3 groups (n = 6 each), and were subjected to the experimental protocol described below, which was approved by Animal Research Committee, Kyoto University.

*UVB irradiation.* The source of UV radiation was a bank of two UV-B F15T8 lamps (Funakoshi, Tokyo). The distance from lamp to dorsal skin was 20 cm. The energy output of the lamps was measured with a UV radiometer. The mice were irradiated 5 times a week for 10 weeks. A progressive UV exposure regimen was used, as follows:[22] The intensity of irradiation was 30 mJ/cm$^2$ (1st week), 50 mJ/cm$^2$ (2nd week), 55 mJ/cm$^2$ (3rd week), 60 mJ/cm$^2$ (4th week), and 65 mJ/cm$^2$ (just below the minimum erythema dose) (5th to 10th weeks). Two h before UVB exposure, the dorsal skin of the experimental mouse was treated with 80 μL of 0.001% fucoxanthin solution or the vehicle control. Fucoxanthin was solubilized in ethanol/propylene glycol/water (3:2:5) for topical application. Unexposed control mice were not treated with any material. At the end of the 10 weeks, the mice were sacrificed by cervical dislocation under

† To whom correspondence should be addressed. Tel/Fax: +81-75-753-6212; E-mail: sugawara@kais.kyoto-u.ac.jp

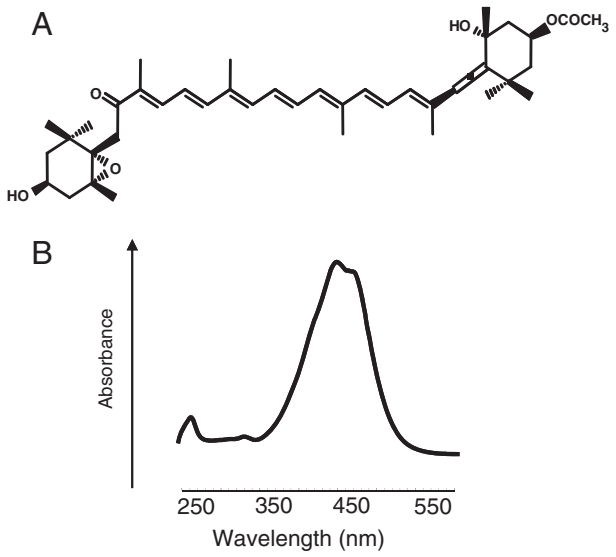


**Fig. 1.** Chemical Structure (A) and UV-VIS Spectrum (B) of Fucoxanthin.

isoflurane anesthesia, and skin specimens were collected immediately. For hematoxylin and eosin staining, dorsal skin samples were excised and fixed in 10% neutral buffered formalin. For analysis of mRNA expression, the skin specimens were stored in RNA later (Qiagen, Valencia, CA) at −80 °C until use. For determination of thiobarbituric acid-reacting substances (TBARS), the specimens were immediately immersed in liquid $N_2$ and stored at −80 °C.

*Evaluation of wrinkle formation in the dorsal skin.* Replicas were taken from the dorsal skin of each mouse using silicon impression material ASA-01W (AsahiBiomed, Tokyo). Images of the replicas were analyzed using an ASA-03RXD based on the guidelines of the Japan Cosmetics Industry Association,[23] and the numbers of wrinkles were calculated per $\mu m^2$.

*Histology.* Skin sections were stained with hematoxylin and eosin. Images were taken with a digital microscope (BIOREVO BZ-9000, Keyence, Osaka, Japan). Images were measured at 10 representative locations for epidermal thickness.

*RNA analysis.* Skin samples in RNA later were washed with HBSS (+) and then incubated with 2.5 U/mL of dispase II in HBSS (+) at 4 °C overnight.[24] After incubation, the epidermis was separated from the dermis at the basement membrane. Total RNA was extracted from the epidermis using Sepasol reagent (Nacalai Tesque, Kyoto, Japan) following the manufacturer's instructions. cDNAs were synthesized using SuperScript RNase II reverse transcriptase (Invitrogen, Carlsbad, CA) with random hexamers. Aliquots (6 µL) of the various cDNAs were used as templates for real-time quantitative PCR (DNA Engine Option System, Bio-Rad Laboratories, Hercules, CA). The following oligonucleotides were used as primers: 5′-CGTCCCGTAGACAAAATGGT-3 and 5′-TGCCGTGAGTGGAGTCATAC-3′ (GAPDH); 5′-TTGTGTTGGGAGGAGGATGT-3′ and 5′-TTGGAACCGGCATCTTTATC-3′ (VEGF); and 5′-CCTGGAATTGGCAACAAAGT-3′ and 5′-CCCACCCCATACATCTGAAA-3′ (MMP-13). The thermocycling conditions were 3 min at 95 °C for one cycle, followed by amplification for 50 cycles with melting for 15 s at 95 °C, and annealing and extension for 30 s at 60 °C. Values were normalized against GAPDH as internal control.

*Determination of TBARS in the skin.*[25] The various skin samples (about 0.5 $cm^2$, 50 ± 1 mg) were cut into pieces in 500 µL of ice-cold 1.15% KCl and homogenized using a homogenizer (T10 basic ULTRA-TURRAX, Boutersem, Belgium) on ice. After 2 freeze-thaw cycles, the samples were cut up again. Each skin homogenate was combined with 500 µL of 15% trichloroacetic acid in 0.25 N HCl and 500 µL of 0.375% thiobarbituric acid in 0.25 N HCl and mixed thoroughly. The solutions were heated for 15 min in a boiling water bath. After cooling, centrifuged at 1,000 × *g* for 10 min and the absorbance was determined at 535 nm against a blank that contained all the reagents minus the tissue. The malondialdehyde concentration of a sample can be calculated using an extinction coefficient of 156,000 $M^{-1}$ $cm^{-1}$.

*Statistical analysis.* Results are expressed as means ± SD. Statistical differences between groups were estimated by one-way analysis of variance (ANOVA) with Fisher's PLSD test, and were considered significant at $p < 0.05$.

## Results

After chronic UVB exposure for 10 weeks, significant wrinkle formation and laxity were observed in the dorsal skin of the mice (Fig. 2). Topical treatment with fucoxanthin before UVB exposure appeared to prevent wrinkle formation judging by skin replica images (Fig. 2D–F). To quantify degrees of wrinkle formation, skin replicas were analyzed using a 3-D image analysis system. The number of wrinkles in the UVB-irradiated skin treated with vehicle alone significantly increased, as compared with the unexposed control (Fig. 2G). Treatment with fucoxanthin had a tendency to suppress the number of wrinkles in the UVB-irradiated dorsal skin of the mice ($p = 0.095$ *vs.* treatment with vehicle), and there was no significant difference between wrinkle formation in the unexposed control group and the fucoxanthin treatment group. The data indicate that topical fucoxanthin treatment contributes to the prevention of wrinkle formation during UVB-induced photoaging.

Epidermal thickness is used as a parameter to reflect skin photoaging quantitatively, since epidermal hypertrophy is thought to cause wrinkle formation. In this study, the epidermal thickness of the dorsal skin of the mice was significantly increased by chronic UVB exposure. This epidermal hypertrophy was significantly reduced by topical application of fucoxanthin prior to UVB exposure (Fig. 3).

To evaluate oxidative stress in the dorsal skin, the levels of TBARS formation were quantified. UVB exposure significantly enhanced the TBARS level in the skin, and topical treatment with fucoxanthin significantly suppressed that increase (Fig. 4A). We proceeded to analyze the effects on mRNA expression of key factors related to ECM degradation and angiogenesis in UVB-irradiated skin. In the case of mice, MMP-13, but not MMP-1, can be related to formation of wrinkles caused by photoaging.[26] Chronic UVB exposure significantly enhanced the expression of MMP-13 and VEGF mRNA, which is known to induce angiogenesis, and topical treatment with fucoxanthin significantly suppressed UVB-induced MMP-13 and VEGF expression in the epidermis (Fig. 4B and C).

## Discussion

The primary mechanism by which UV exposure initiates molecular responses in the skin is by the photochronical generation of ROS.[2] ROS causes structural and functional alterations of the ECM, and also causes direct deleterious chemical modifications to cellular components. In addition, ROS activate cell

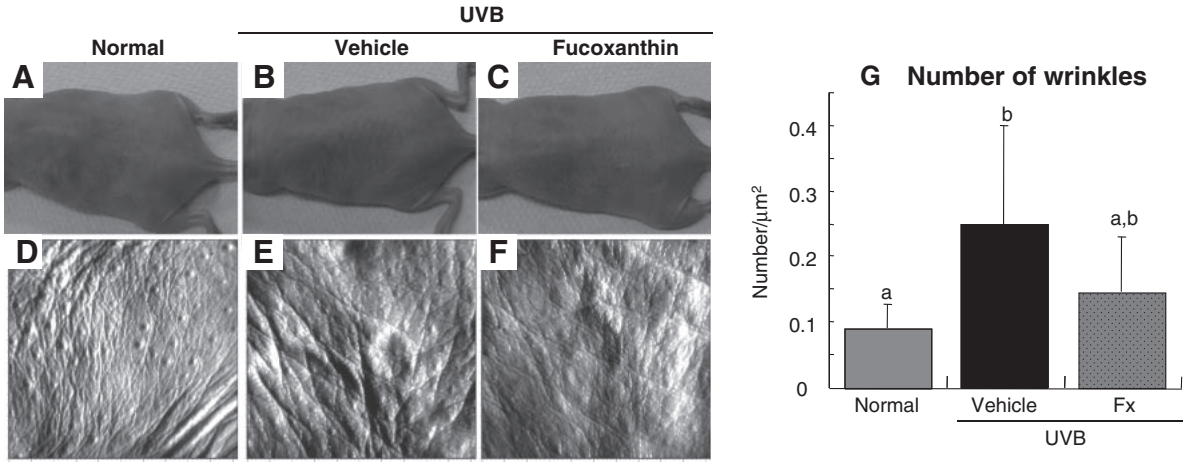


**Fig. 2.** Effects of Topical Application of Fucoxanthin on UVB-Induced Wrinkle Formation.

Features of hairless mouse dorsal skin (A–C) and photographs of replicas taken from the dorsal skins of the mice (D–F) after UVB irradiation. Untreat, unexposed normal mice (A, D); UVA-irradiate, vehicle-treated control mice (B, E); UVB-irradiate 0.001% fucoxanthin-treated mice (C, F). Photographs of replicas taken from hairless mouse dorsal skin were analyzed by the 3-D image analysis system (G). Values are means ± SD (n = 6). Bars with different letters are significantly different from each other ($p < 0.05$).

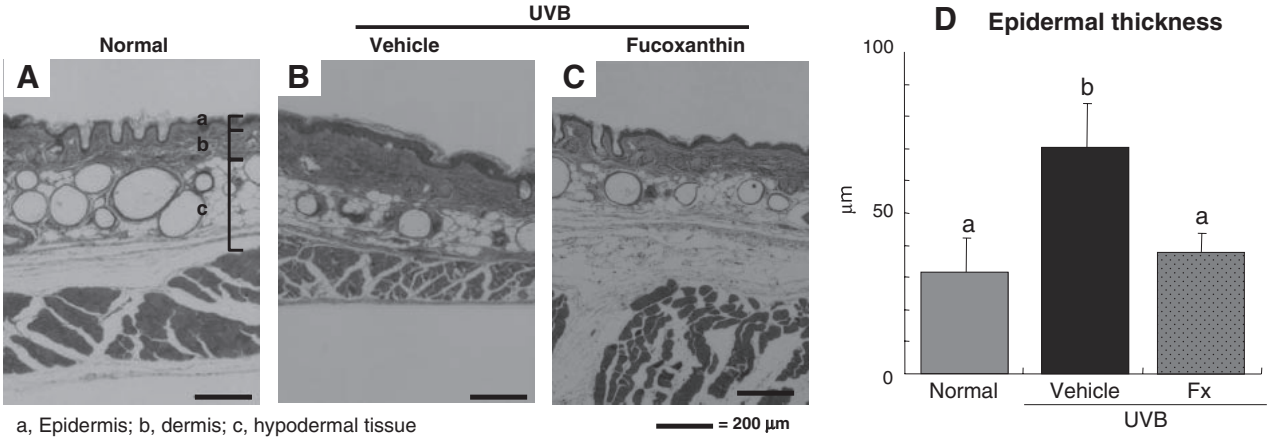


**Fig. 3.** Effect of Topical Application of Fucoxanthin on UVB-Induced Epidermal Hypertrophy.

Skin sections were stained with hematoxylin and eosin (A–C). Untreat, unirradiated normal mice (A); UVB-irradiate, vehicle-treated control mice (B); UVB-irradiate 0.001% fucoxanthin-treated mice (C). Magnification, ×200. The epidermal thicknesses of the various specimens were quantified by microscopy (D). Values are means ± SD (n = 6). Bars with different letters are significantly different from each other ($p < 0.05$).

surface cytokines and growth factor receptors in the skin. The activated receptors can stimulate signal transduction cascades that induce expression of transcription factor AP-1, which in turn stimulates the transcription of matrix metalloproteinases (MMPs). MMPs break down the ECM and cause dermal damage that eventually results in the characteristic wrinkling of photodamaged skin. Hence, the use of antioxidants is believed to be an effective approach to preventing the symptoms related to photoaging of the skin.[27] Carotenoids are among the most efficient natural scavengers of singlet molecular oxygen, and at low oxygen tension carotenoids also scavenge peroxyl radicals. Fucoxanthin shows radical-scavenging and singlet-quenching activities.[15] In addition, a protective effect of fucoxanthin against UVB-induced oxidative stress in human fibroblasts has been reported.[16] Thus, fucoxanthin can act as a potent antioxidant in the skin. It reduced UV-induced ROS and the expression of MMP-13 in this study.

Angiogenesis, the process of generating new blood vessels, is also affected by various physiological and pathological conditions of the skin. VEGF, a potent angiogenic factor, is upregulated by UV exposure of the skin.[6,28] The level of VEGF is an important parameter in maintaining balanced skin angiogenesis. Yano *et al.* have reported that angiogenesis in the skin plays a critical role in photoaging, and that inhibition of angiogenesis diminishes UV-induced wrinkle formation.[5] In addition, they found that VEGF can promote sensitivity to UVB-induced cutaneous photodamage.[7]

Our results indicate that the topical application of fucoxanthin suppresses UV-induced VEGF expression in the skin. The suppressive effect of VEGF may be important in the prevention of skin photoaging, including wrinkling by fucoxanthin. We have found that fucoxanthin strongly prevents angiogenesis, as evaluated using cultured human umbilical vein endothelial cells and the rat aortic ring.[20] The antioxidant activity of fucoxanthin might be involved in this antiangiogenic effect, because ROS stimulate angiogenesis.[29,30] The details of the molecular mechanism of the antiangiogenic effect of fucoxanthin in UV-exposed skin should be addressed in order to determine the mechanism of anti-photoaging.

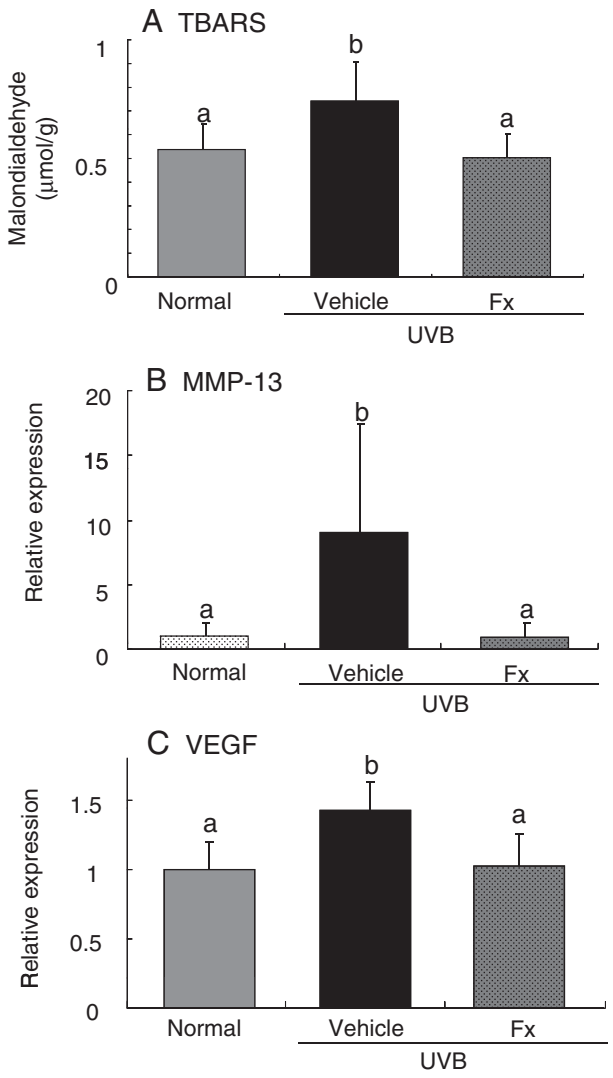


**Fig. 4.** Effect of Topical Application of Fucoxanthin on the TBARS (A) and mRNA Expression Levels of MMP-13 (B) and VEGF (C) in the Epidermis.

Values are means ± SD (n = 6). Bars with different letters are significantly different from each other ($p < 0.05$).

In conclusion, we found that topical treatment with fucoxanthin prevented photoaging as evidenced by wrinkle formation and epidermal hypertrophy in the skin of UVB-irradiated hairless mice, possibly *via* its antioxidant and antiangiogenic effects. This finding might be useful in the exploitation of fucoxanthin in cosmetics. It is not likely that the sunscreen action of fucoxanthin played a major role in its protective effect against UV-induced photoaging, because the UVB absorption (290–320 nm) of fucoxanthin is relatively weak. Because fucoxanthin is metabolized to fucoxanthinol and amarouciaxanthin A after oral ingestion,[31,32] it will be interesting to evaluate the effect of dietary fucoxanthin on skin photoaging.

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