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Research Paper

Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules

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Abstract

Objectives Carotenoids and retinoic acid derivatives are topically applied for sun-protective and whitening purposes. Fucoxanthin is a carotenoid derived from edible sea algae, but its effect on melanogenesis has not been established. Therefore, we examined the effect of fucoxanthin on melanogenesis.

Methods Inhibitory effects on tyrosinase activity, melanin formation in B16 melanoma and skin pigmentation in UVB-irradiated guinea-pigs were evaluated. To elucidate the action of fucoxanthin on melanogenesis, its effect on skin melanogenic mRNA expression was evaluated in UVB-irradiated mice. Fucoxanthin was given topically or orally to mice once a day and UVB irradiation was applied for 14 days. The effect of fucoxanthin on skin melanogenic mRNA expression was evaluated by real time reverse transcription polymerase chain reaction.

Key findings Fucoxanthin inhibited tyrosinase activity, melanogenesis in melanoma and UVB-induced skin pigmentation. Topical application of fucoxanthin (1%) significantly suppressed mRNA expression of cyclooxygenase (COX)-2, endothelin receptor A, p75 neurotrophin receptor (NTR), prostaglandin E receptor 1 (EP1), melanocortin 1 receptor (MC1R) and tyrosinase-related protein 1. The suppression of p75NTR, EP1 and MC1R expressions was observed at 0.01% application. Also, oral application of fucoxanthin (10 mg/kg) significantly suppressed expression of COX-2, p75NTR, EP1 and MC1R.

Conclusions These results suggest that fucoxanthin exhibits anti-pigmentary activity by topical or oral application in UVB-induced melanogenesis. This effect of fucoxanthin may be due to suppression of prostaglandin (PG) E_2 synthesis and melanogenic stimulant receptors (neurotrophin, PGE_2 and melanocyte stimulating hormone expression).

Keywords endothelin; fucoxanthin; melanin; melanocortin; prostaglandin; tyrosinase-related protein

Introduction

Recently, preventive effects of naturally occurring carotenoids on UV-induced skin photoaging and tissue damage have been elucidated.[1] For example, dietary supplementation of lutein and zeaxanthin contained in marigold flowers reduced photoaging and photocarcinogenesis in UVB-irradiated mouse skin.[2] Reduction of acute inflammatory responses and hyperproliferation of skin cells are involved in the inhibitory mechanism.[3] These carotenoids also suppress lipid peroxidation and exhibit photoprotective activity in UV-irradiated human skin following oral and topical application.[4] β-Cryptoxanthin contained in citrus fruits distributes to skin after ingestion and affects skin hydration.[5] Moreover, astaxanthin contained in algae has been reported to exhibit potent photoprotective activity in human fibroblasts.[6]

Many reports have described the photoprotective activity of dietary carotenoids, which seems to be based on their anti-oxidative activity. However, only a few reports have described the anti-melanogenic effect of carotenoids. All-*trans* retinoic acid (atRA), a synthetic vitamin A derivative, has been topically applied for skin pigmentation in clinical therapy.[7] Topical application of atRA is well recognized as a potent retinoid therapy for skin pigmentary disorders[8] and solar lentigines.[9] In contrast to the efficacy of atRA, its teratogenicity on embryonic and prenatal development have been reported.[10,11] Moreover, atRA was reported to augment skin photocarcinogenesis in UV-irradiated mice.[12] Hence, exploration of the

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safety carotenoids with efficacy for skin pigmentation is considered to be important in clinical and cosmetic fields.

Fucoxanthin is a carotenoid contained in brown algae such as Kombu (*Laminaria japonica*) and Wakame (*Undaria pinnatifida*). Previous reports have described its biological actions, including anti-cancer,[13] anti-obesity,[14] anti-angiogenic[15] and anti-allergic[16] activities. However, regarding the action of fucoxanthin on skin, only one study has reported that fucoxanthin suppressed UV-induced cell injury in human fibroblasts.[17] Tyrosinase is a key enzyme on melanogenesis[18] and inhibitory activity of the enzyme is well used as an index for evaluation of the anti-melanogenic activity of compounds. In addition, a recent report has proposed that a composition of compounds with different suppressive mechanisms was effective for melanin synthesis.[19] In skin melanogenesis, substances released from epidermal cells include melanocyte-stimulating hormone (MSH), endothelins, neurotrophins and prostaglandins.[20,21] The receptors of these cytokines exist on melanocytes and they transmit the signals leading to melanogenesis. From this viewpoint, investigation of the actions of fucoxanthin on melanogenesis is significant.

Therefore, we evaluated the effect of fucoxanthin on melanin synthesis *in vitro* and *in vivo* and found that fucoxanthin suppressed melanogenesis. Moreover, topical and oral application of fucoxanthin to UVB-irradiated mice suppressed skin mRNA expression related to melanogenesis. In this paper, we describe the effect of fucoxanthin on skin melanogenesis and changes in skin mRNA expression.

Materials and Methods

Reagents

L-DOPA was obtained from Acros Organics (Geel, Belgium). Tyrosinase (EC1.14.18.1, from mushroom), arbutin, Eagle's minimum essential medium (EMEM) and theophylline were purchased from Sigma-Aldrich (St Louis, USA). An RNeasy Protect Mini Kit was purchased from Qiagen (Hilden, Germany). Fetal calf serum (FCS), random hexamers, 10 mM dNTP mixture (PCR grade) and an RNase inhibitor were obtained from Invitrogen Co. (Carlsbad, USA). PrimeScript Reverse Transcriptase and SYBR Premix Ex Taq were purchased from Takara Bio Inc. (Otsu, Japan).

Preparation of fucoxanthin

Kombu (*Laminaria japonica*) harvested (in May 2008) in the Hakodate area in Japan was stocked at −20°C until extraction. Thawed Kombu (3.5 kg) was cut into small pieces (approximately 3 cm square) and extracted with ethanol (20 l) for 1 h at 40°C. The extracted solution was evaporated to obtain the ethanolic extract (26.6 g). The extract was partitioned with ethanol (100 ml × 3) and the ethanol portion was evaporated to obtain a desalted fraction (10.6 g). The desalted fraction was washed with *n*-hexane (100 ml × 3) to exclude chlorophyll. The washed fraction was separated by silica gel column chromatography (silica gel, 200 g) with ethanol to obtain a crude fucoxanthin fraction (1.6 g). The fraction was repeatedly purified by an HPLC column (Inertsil prep ODS, 20 mm i.d. × 250 mm; GL Science, Tokyo, Japan) with acetonitrile–methanol–H_2O (60 : 25 : 15) as a solvent. The wavelength of the UV-visible detector was set at 450 nm and dark red powder (480 mg) was isolated with a yield of 0.013%. The powder was identified as fucoxanthin by comparing the ^{13}C and ^{1}H NMR spectra with those value of an authentic sample. The content of fucoxanthin in the dark red powder was determined by HPLC equipped with a C18 column, 4.6 mm i.d. × 250 mm (Capcell pack C18, SG120; Shiseido Co. Ltd, Tokyo, Japan). The flow rate was fixed at 1 ml/min and acetonitrile–methanol–H_2O (60 : 25 : 15) was used as an eluant. The wavelength for detection was 450 nm and the column was kept at 35°C. The content of fucoxanthin in the dark red powder was 92.0%. The powder was used as fucoxanthin for subsequent experiments.

Animals and cells

Guinea-pigs (Weiser-Maples, male, 4 weeks old) were purchased from Kiwa Laboratory Animals Co. Ltd (Wakayama, Japan). Male hairless mice (Hos; HRM2, 4–5 weeks old) were obtained from Hoshino Laboratory Animals Inc. (Yashio, Saitama, Japan). The animals were housed in an air-conditioned room (23 ± 1°C, 50 ± 10% relative humidity) for three or more days. RC-4 (Oriental Yeast Co. Ltd, Tokyo, Japan) for guinea-pigs or CE-2 (Clea Japan Inc., Shizuoka, Japan) for mice and tap water were given freely. The experiments were performed in accordance with the Guidelines for Animal Experimentation (Japan Association for Laboratory Animal Science, 1987). Mouse B16 melanoma (JCRB0202) was obtained from the Health Science Research Resources Bank (Sennan, Osaka, Japan). All experiments were approved by the ethical committee of research and development section in Oryza Oil & Fat Chemical Co. Ltd.

Effect of fucoxanthin on tyrosinase activity and melanogenesis in melanoma

For the tyrosinase assay, fucoxanthin diluted in dimethyl sulfoxide (DMSO, 40 μl) was added to a mixture of 40 mM phosphate-buffered saline (PBS, 1360 μl, pH 6.8) and 0.4 mg/ml L-DOPA (500 μl). One hundred microlitres of tyrosinase (300 U/ml) was added and incubated for 5 min at room temperature. The absorbance of reacted solution was measured at 490 nm referring to the absorbance of a mixture without tyrosinase as a blank.

Evaluation of the effect of fucoxanthin on melanogenesis in B16 melanoma was performed with the following procedure. Cells pre-cultured in EMEM containing 10% FCS, penicillin (100 U/ml) and streptomycin (100 μg/ml) were collected and suspended in a medium containing 2 mM theophylline. The cells (2.5×10^5 cells/500 μl) were seeded in a 24-well culture plate and cultured for 2 h. Fucoxanthin or arbutin dissolved in 10% DMSO (55 μl) was added and cultured for three days. As a control group, 10% DMSO (55 μl) was added to the cells. The medium was removed and 200 μl of PBS was added to the well. The cells were sonicated and the cell suspension (100 μl) was collected in a 96-well plate. The absorbance of the cell suspension was measured at 415 nm toward 700 nm as the reference wavelength.

Animal experiments

To examine its effect on UV-induced skin pigmentation in guinea-pigs, fucoxanthin was mixed with a non-purified diet

at a concentration of 0.001%. The diet was given freely to guinea-pigs during the breeding period (day – 10 to 15). After feeding for seven days, UVB (2000 mJ/cm^2) was irradiated to the shaved back skin in guinea-pigs once a day for three consecutive days (day – 2 to 0) using a UV irradiator (Solar Simulator; Ushio Inc., Tokyo, Japan). Lightness (L* value) of the irradiated spots on the skin was measured using a spectrocolorimeter (SE2000; Nippon Denshoku Industries Co. Ltd, Tokyo, Japan) on days – 3, 0, 4, 6, 8, 10, 13 and 15.

To examine the effect of topical and oral treatment of fucoxanthin on skin mRNA changes, hairless mice were used. For topical treatment, fucoxanthin was mixed with white petrolatum (0.01, 0.1 and 1%). The ointment (50 μl) was applied once a day to the back skin of the mice shortly after UVB irradiation. For oral treatment, fucoxanthin was suspended in water (0.1, 1 and 10 mg/kg) with 5% acacia and given once a day, 2 h before UVB irradiation. UVB (160 mJ/cm^2) was irradiated to back skin for seven days followed by increased UVB (320 mJ/cm^2) irradiation for seven more days. On the day after the last UVB irradiation, the irradiated skin area was removed and the specimens were soaked in 10% neutralized formaldehyde or 'RNA later' attached to an RNeasy Protect Mini Kit, respectively. Each specimen soaked in formaldehyde was stained by Fontana-Masson staining for melanin detection. Microscopic observation was performed at a magnification of × 400. The specimens in 'RNA later' were stored at 4°C for RT-PCR analysis.

Real time reverse transcription-polymerase chain reaction (RT-PCR) analysis of skin mRNA expression related to melanogenesis

Each skin specimen (approximately 30 mg) was extracted and purified by the RNeasy Mini Kit to obtain total RNA. The cDNA was synthesized from 0.1 μg of total RNA by random hexamers, dNTP mixture, PrimeScript Reverse Transcriptase and an RNase inhibitor. RT-PCR was performed by a Thermal Cycler Dice Real Time System (TM800; Takara Bio Inc., Shiga, Japan) using cDNA (3.4 ng), SYBR Premix Ex Taq and the following primers. Primers (5′→3′) used were endothelin (ET)-1: forward: CCTGGACATCATCTGGGTC, reverse: TGTGGCCTTATTGGGAAG, neurotrophin (NT)-3: forward: CATGTCGACGTCCCTGGAAATAG, reverse: TGGACA TCACCTTGTTCACCTGTAA, cyclooxygenase (COX)-2: forward: CAGTCAGGACTCTGCTCACGAA, reverse: AGCAGCACAGCTCGGAAGA, proopiomelanocortin (POMC): forward: CGCCCGTGTTTCCA, reverse: TGA CCCATGACGTACTTCC, ET receptor A (EDNRA): forward: GGTGGCTCTTTGGGTTCT, reverse: GACGCT GTTTGAGGTGCT, NT-3 receptor (NT3R): forward: GCCAAGTGTAGTTTCTGGCG, reverse: CAGACACAA TTTGCAGGGCA, p75 NT receptor (p75NTR): forward: CTAGGGGTGTCCTTTGGAGGT, reverse: CAGGGTTCA CACACGGTCT, prostaglandin E receptor 1 (EP1): forward: GGGCTTAACCTGAGCCTAGC, reverse: GTGATGTGCCA TTATCGCCTG, melanocortin 1 receptor (MC1R): forward: GACCGCTACATCTCCATCTTCT, reverse: AGGAGGAG GAAGAGGTTGAAGT, tyrosinase (Tyr): forward: TCAT TGTGAATTTCCAAGAAAAA, reverse: GCTGGCAGAT GTTCTCCTCT, tyrosinase related protein 1 (Tyrp1): forward: GCATTGCTCTCCAGTGATGA, reverse: TTTTCACAG

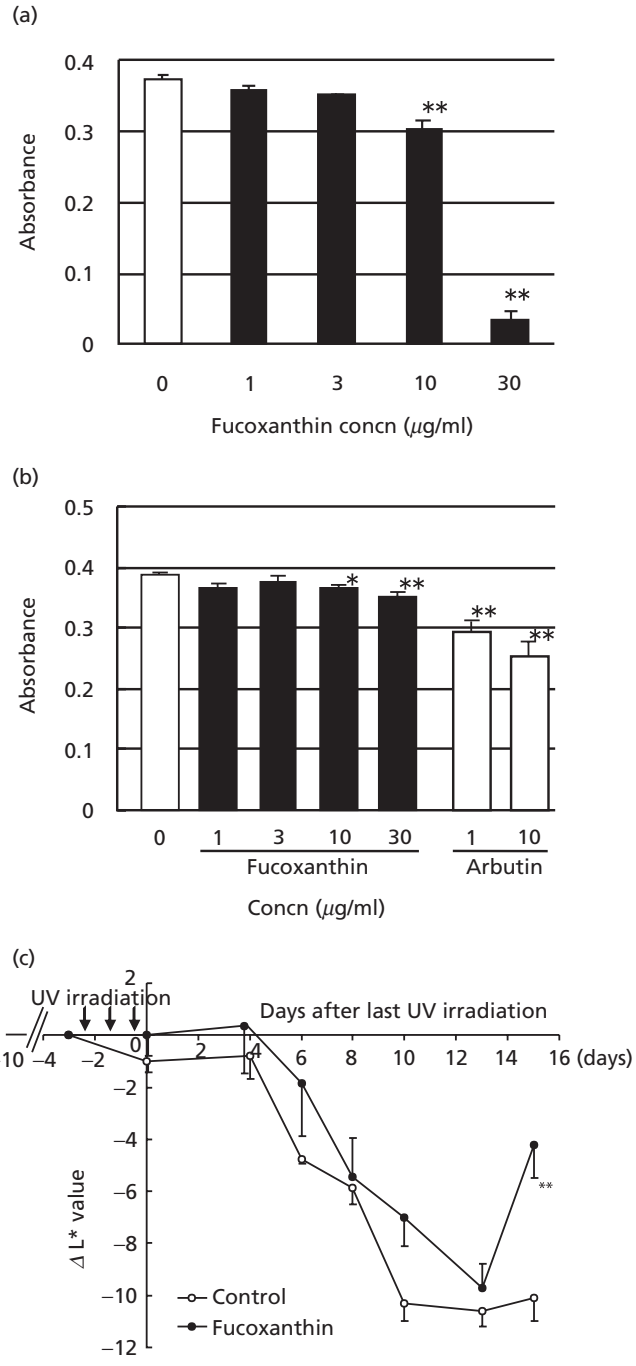

Figure 1 Suppressive effect of fucoxanthin on (a) tyrosinase activity, (b) melanogenesis in melanoma and (c) pigmentation in UV-irradiated guinea-pigs. (a) Each column represents the mean with the SE, $n = 3$. (b) Each column represents the mean with the SE, $n = 4$. (c) Each points represents the mean with the SE, $n = 4$. *$P < 0.05$, **$P < 0.01$, compared with control, for all Figures.

CATACAGGCCA, β-actin: forward: AATCGTGCGTGA CATCAAAG, reverse: GAAAAGAGCCTCAGGGCAT. The cycling conditions were as follows: one denaturing cycle at 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 30 s, dissociation step at 95°C for 15 s, 60°C for 30 s and 95°C for 15 s. Relative quantification was calculated using the

$\Delta\Delta$Ct (threshold cycle) method, where ΔCt is (target Ct – β-actin Ct), and $\Delta\Delta$Ct is (ΔCt sample – ΔCt untreated control). Relative quantity is $2^{-\Delta\Delta Ct}$. Each mRNA expression was indicated with a Ct value and corrected using the expression of β-actin.

Statistics

The results are expressed as means and SE. Significance of the differences was examined by one-way analysis of variance followed by Dunnett's test for multiple groups. Student's *t*-test was used for the result obtained in guinea-pigs. $P < 0.05$ was considered significant.

Results

Suppression of tyrosinase activity and melanogenesis by fucoxanthin

Figure 1a shows the effect of fucoxanthin on tyrosinase activity. Fucoxanthin significantly inhibited tyrosinase activity at 10 and 30 μg/ml. Regarding the melanogenesis in B16 melanoma induced by theophylline, fucoxanthin slightly suppressed melanogenesis at 10 and 30 μg/ml by three-day treatment (Figure 1b). Arbutin used as positive control significantly suppressed melanogenesis at 1 and 10 μg/ml. On the other hand, as for the UVB-induced skin pigmentation in guinea-pigs, lightness (L* value) decreased from six days after the last UVB irradiation (Figure 1c). This reduction means formation of pigmentation. The reduction in the L* value continued until 10 days after the UVB irradiation and reached a plateau for the control. By continuous feeding of the diet containing 0.001% fucoxanthin, the L* value decreased, but maintained a slightly higher value compared with the control group. The L* value of the guinea-pigs treated with fucoxanthin on day 15 exhibited a significantly higher value than that of the control.

Microscopic findings of the epidermis in UV-irradiated mice treated with fucoxanthin

Figure 2 shows microscopic images of skin of the UV-irradiated mice topically applied with or without fucoxanthin. In the control image (Figure 2a), melanogenesis was observed in the epidermis. In mice treated with fucoxanthin (0.01, 0.1 and 1%), a reduction in melanogenesis was observed

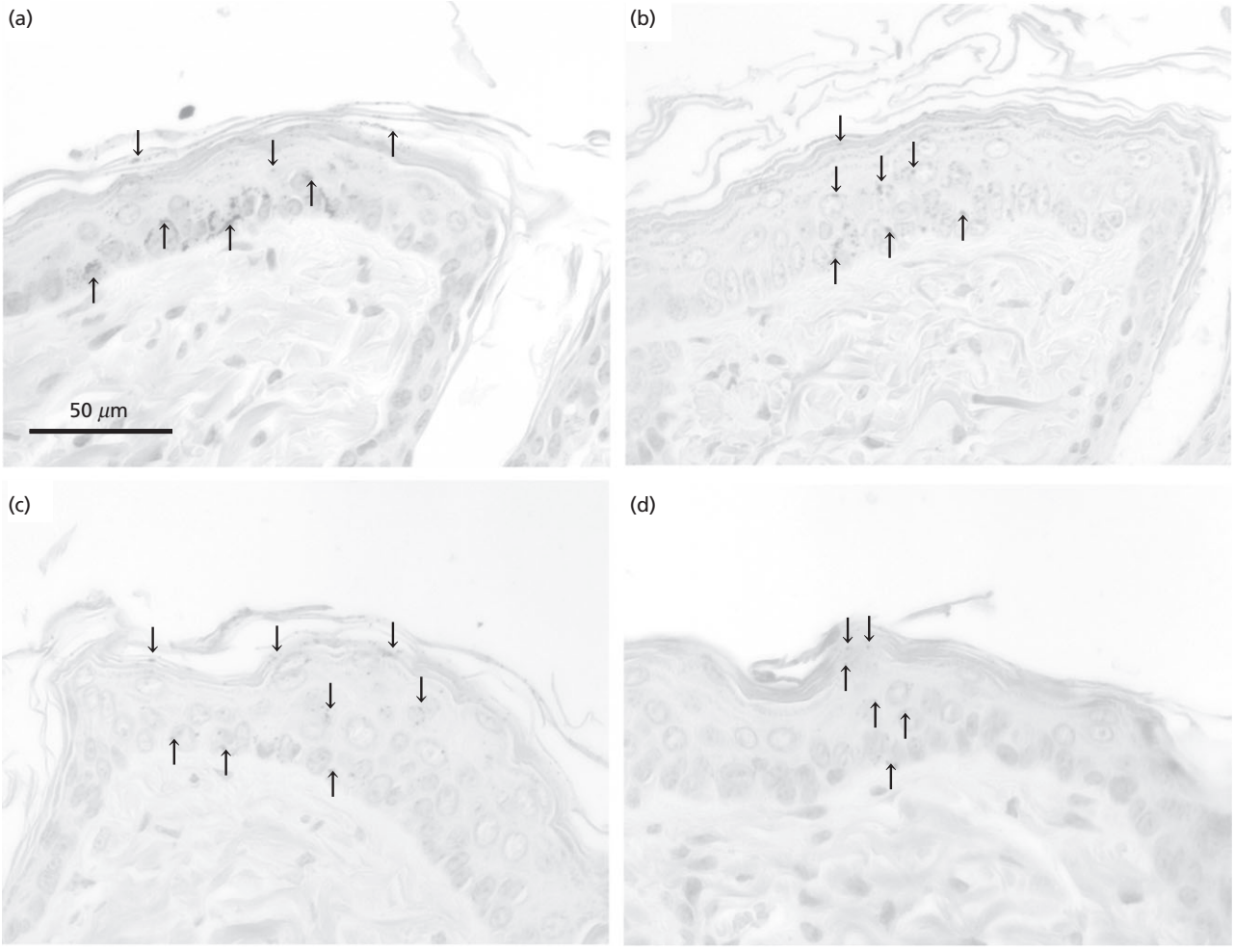

Figure 2 Microscopic images of a cross-section of skin topically treated with fucoxanthin. (a) Control, (b–d) ointments containing 0.01, 0.1 and 1% fucoxanthin. Arrows indicate melanin.

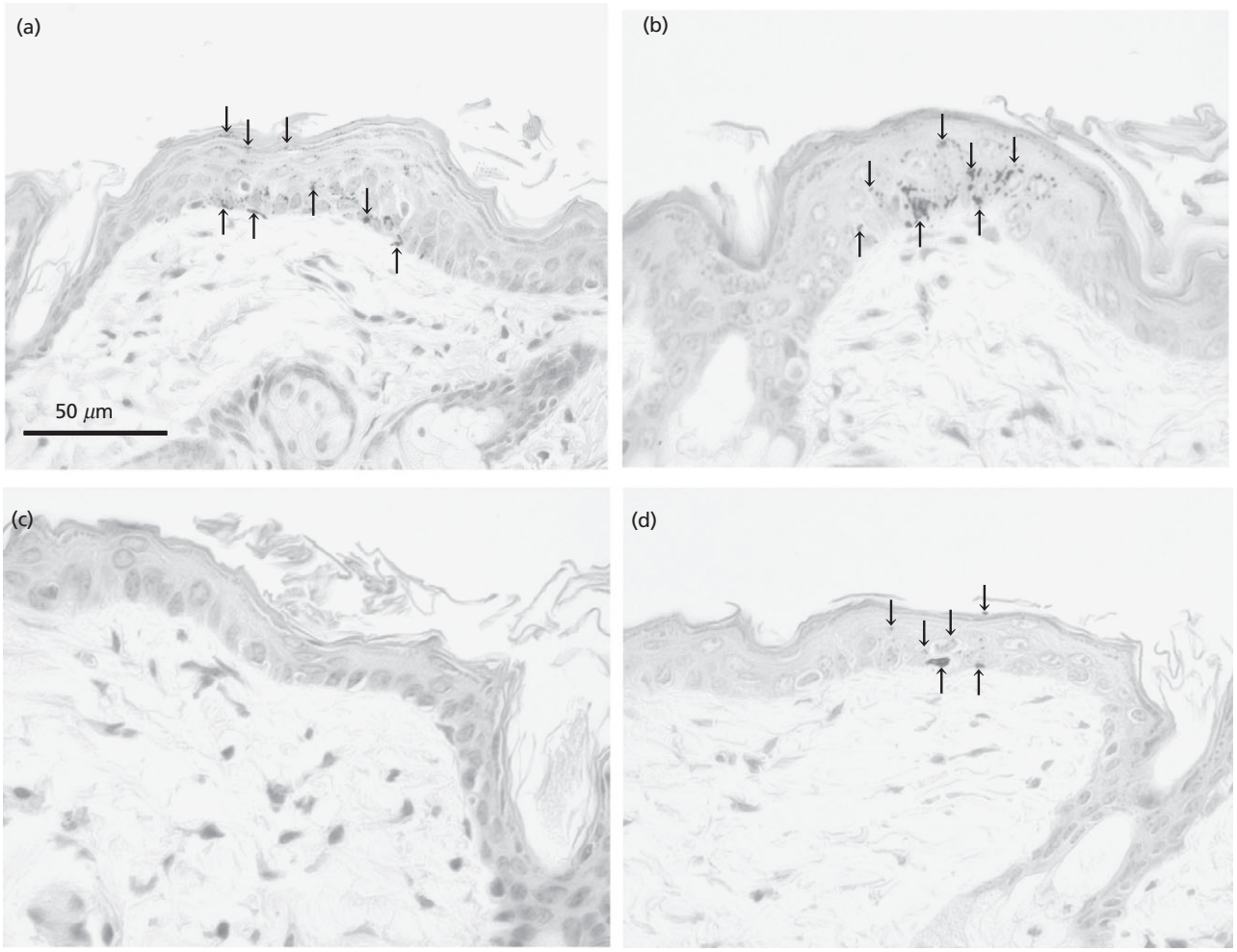

Figure 3 Microscopic images of a cross-section of skin orally treated with fucoxanthin. (a) control, (b–d) 0.1, 1 and 10 mg/kg fucoxanthin. Arrows indicate melanin.

(Figure 2b–d). Figure 3 shows images of the skin of mice orally given fucoxanthin. Compared with the control (Figure 3a), a decrease in melanin was observed in the images of the mouse skin treated with 1 and 10 mg/kg fucoxanthin (Figure 3c–d).

Effect of fucoxanthin on skin mRNA expression related to melanogenesis in UV-irradiated mice

Table 1 shows the expression ratio of mRNA related to skin melanogenesis. We chose mRNAs of POMC, ET-1, NT-3 and COX-2 as markers of activation of epidermal cells. However, mRNA expression of POMC, a precursor of MSH, was not detected in control. By topical treatment with ointment containing 1% fucoxanthin, mRNA expression of COX-2, which synthesizes prostaglandin in epidermal cells, was significantly suppressed. mRNA expression of ET-1 and NT-3 released from keratinocytes was not suppressed. Various receptors of melanogenic stimulants, namely receptors of ET (EDNRA), NT (NT3R, p75NTR), PG (EP1) and MSH (MC1R), exist on melanocytes. Binding signals of the stimulants are transmitted into the nucleus and mRNA of tyrosinase (Tyr) and tyrosinase related protein (Tyrp1), which play a direct role in melanogenesis, are synthesized. As for the expression of receptors that bind stimulants for melanogenesis and melanogenic enzymes, EDNRA, p75NTR, EP1, MC1R and Tyrp1 were significantly suppressed by 1% fucoxanthin treatment. Tyrosinase was slightly suppressed, but this was not significant. Inhibition of the expression of p75NTR, EP1 and MC1R was observed on treatment with 0.01% fucoxanthin. The mRNA expression of NT-3 and NT3R was significantly enhanced by treatment with 0.1% fucoxanthin. On the other hand, oral treatment with fucoxanthin (10 mg/kg) significantly suppressed expression of COX-2, p75NTR, EP1 and MC1R (Table 2). MC1R and Tyrp1 tended to be suppressed without significance. The mRNA expression of ET-1 and NT-3R was significantly enhanced by treatment with fucoxanthin (0.1–10 and 1 mg/kg, respectively).

Discussion

As a basic effect on melanogenesis, fucoxanthin (10 and 30 μg/ml (15.1 and 45.5 μM)) was found to suppress tyrosinase activity and cellular melanogenesis. In contrast to the tyrosinase inhibitory activity of fucoxanthin, suppression of

Table 1 Effect of topical treatment with fucoxanthin on skin mRNA expression in UVB-irradiated mice

	Ct of control	Control	Fucoxanthin (%)		
			0.01	0.1	1
Released cytokine from epidermal cell					
ET-1	27.2	1.00 ± 0.02	1.12 ± 0.18	1.23 ± 0.09*	0.97 ± 0.11
NT-3	30.7	1.00 ± 0.02	1.84 ± 0.13	2.98 ± 0.07**	1.16 ± 0.05
COX-2	27.1	1.00 ± 0.01	1.01 ± 0.22	0.85 ± 0.18	0.73 ± 0.05*
Receptor in melanocyte					
EDNRA	25.3	1.00 ± 0.01	1.03 ± 0.08	0.98 ± 0.11	0.83 ± 0.05*
NT3R	28.7	1.00 ± 0.02	0.98 ± 0.09	1.29 ± 0.24**	0.81 ± 0.14
p75NTR	27.8	1.00 ± 0.04	0.70 ± 0.08**	0.70 ± 0.18*	0.57 ± 0.16**
EP1	27.7	1.00 ± 0.02	0.73 ± 0.10**	0.86 ± 0.16	0.66 ± 0.07**
MC1R	27.2	1.00 ± 0.05	0.79 ± 0.02**	0.75 ± 0.03**	0.66 ± 0.03**
Melanogenesis in melanocyte					
Tyr	26.3	1.00 ± 0.03	0.91 ± 0.07	0.85 ± 0.16	0.77 ± 0.15
Tyrp1	25.0	1.00 ± 0.03	0.85 ± 0.11	0.74 ± 0.08**	0.64 ± 0.11**

Each value represents the mean ± SE of five mice. *$P < 0.05$, **$P < 0.01$, compared with control. Each mRNA expression was corrected by that of β-actin.

Table 2 Effect of oral treatment with fucoxanthin on skin mRNA expression in UVB-irradiated mice

	Ct of control	Control	Fucoxanthin (mg/kg)		
			0.1	1	10
Released cytokine from epidermal cell					
ET-1	27.3	1.00 ± 0.01	1.23 ± 0.10*	1.40 ± 0.13**	1.27 ± 0.16*
NT-3	29.3	1.00 ± 0.02	0.90 ± 0.05	0.82 ± 0.06	0.86 ± 0.01
COX-2	27.9	1.00 ± 0.01	0.86 ± 0.16	0.89 ± 0.11	0.74 ± 0.09*
Receptor in melanocyte					
EDNRA	25.5	1.00 ± 0.01	0.96 ± 0.04	1.09 ± 0.05	0.96 ± 0.06
NT3R	28.8	1.00 ± 0.02	1.01 ± 0.16	1.24 ± 0.11*	0.96 ± 0.10
p75NTR	28.9	1.00 ± 0.04	0.84 ± 0.18	0.87 ± 0.06	0.69 ± 0.07**
EP1	28.2	1.00 ± 0.01	1.05 ± 0.08	0.89 ± 0.07	0.80 ± 0.09*
MC1R	25.5	1.00 ± 0.01	0.94 ± 0.17	0.87 ± 0.08	0.76 ± 0.06*
Melanogenesis in melanocyte					
Tyr	27.1	1.00 ± 0.03	0.99 ± 0.16	1.04 ± 0.14	0.96 ± 0.09
Tyrp1	28.0	1.00 ± 0.03	0.95 ± 0.20	1.01 ± 0.19	0.69 ± 0.16

Each value represents the mean ± SE of five mice. *$P < 0.05$, **$P < 0.01$, compared with control. Each mRNA expression was corrected by that of β-actin.

melanogenesis in B16 melanoma was weak. Incorporation of fucoxanthin into the cells in an in-vitro culture system may be difficult. In B16 melanoma stimulated by MSH, Sato *et al*.[22] reported that atRA (5 μM) and retinol (10 μM) inhibited melanogenesis through suppression of Tyr and Tyrp1, respectively. Roméro *et al*.[23] reported that expression of Tyr and Tyrp1 was suppressed by RA (1 μM) in UVB-irradiated S91 mouse melanoma. The suppressive effect of fucoxanthin on melanogenesis in melanoma seemed to be half that of atRA. From these reports and our findings, fucoxanthin is suggested to exhibit a weak inhibitory activity on melanogenesis via suppression of Tyr and Tyrp1. On the other hand, from the results of our experiment in guinea-pigs, daily intake of low-dose fucoxanthin (0.001% in diet) suppressed UVB-induced skin pigmentation. Ingested fucoxanthin was reported to be present in the blood as a decarboxylate product (fucoxanthinol) and further an epoxy cleavage (amarouciaxanthin A). The metabolites distribute to the liver, heart and adipose tissue.[24] Fucoxanthin accumulates in the heart and liver as fucoxanthinol and in adipose tissue as amarouciaxanthin A. The metabolite in skin has not yet been clarified, but, these two fucoxanthin metabolites are highly likely to be accumulated in mouse skin and suppress melanin synthesis.

From the microscopic analysis of UVB-irradiated mouse skin, production of melanin was observed in the lower part of epidermis. Topical (0.01–1%) and oral (1 and 10 mg/kg) treatment with fucoxanthin suppressed melanin accumulation. To investigate molecules affected by fucoxanthin in melanogenesis, we analysed mRNA expression in epidermal cells and melanocytes. Skin epidermal cells produce ET-1,[25] POMC,[26] NT-3[27] and PGE_2[28]. UVB enhances production of these mediators leading to melanogenesis.[29,30] Topical (1%) and oral (10 mg/kg) treatment with fucoxanthin suppressed mRNA expression of COX-2, which synthesizes PGE_2. β-Carotene, known as an anti-oxidative and anti-inflammatory carotenoid, was reported to suppress mRNA expression of COX-2 in cultured human keratinocytes.[31] The suppressive effect of

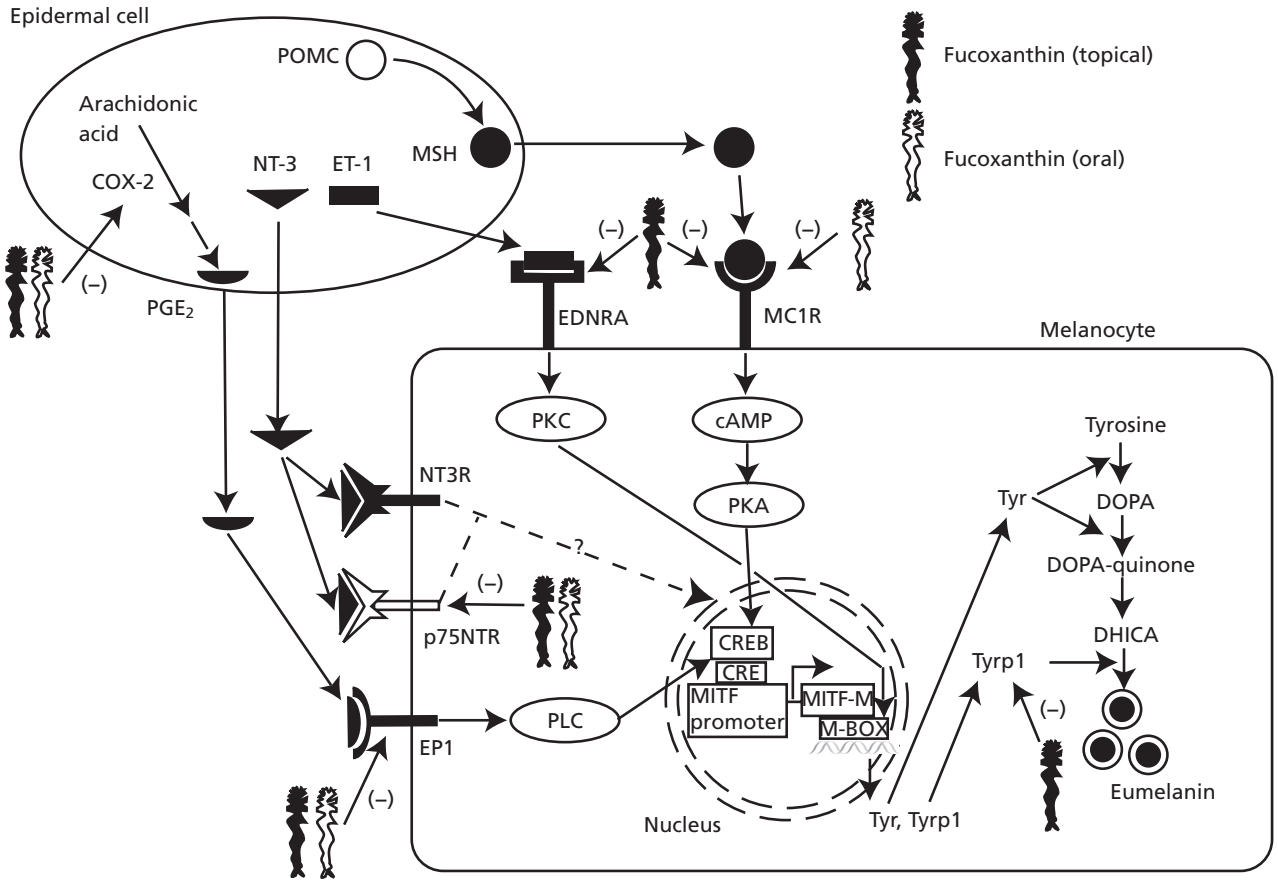

Figure 4 Schematic pathway of melanogenesis and molecules affected by fucoxanthin. The pathway of melanogenesis was cited and summarized from the report of Costin and Hearing.[20] CRE, cAMP response element; CREB, CRE binding protein; DHICA, 5,6-dihydroxyindole-2-carboxylic acid; MITF, microphthalmia-associated transcription factor; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C.

fucoxanthin seems to be similar to β-carotene. On the other hand, ET-1 expression was slightly enhanced by topical and oral treatment with fucoxanthin. Yokota *et al.* [32] reported that atRA suppressed mRNA expression of ET-1 in cultured endothelial cells and a retinoic acid receptor (RAR) antagonist (LE-540) reversed this effect. Hence, fucoxanthin may act as antagonist on RAR in epidermal cells and dermal microcapillary vessels, enhancing mRNA expression of ET-1. mRNA expression of NT-3 was also up-regulated by topical treatment with fucoxanthin. Although NT-3 stimulates melanogenesis,[21] it also enhances neurogrowth. Retinoic acid is recognized to enhance neuronal differentiation[33] and neurite outgrowth[34]. Thus, fucoxanthin may exhibit retinoid-like activity and enhance NT-3 expression in epidermal cells and neuronal cells distributed in skin.

Regarding mRNA expression of receptors in melanocytes related to melanogenesis, topical application of fucoxanthin (1%) significantly suppressed EDNRA, p75NTR, EP1 and MC1R. EDNRA is an ET-1 receptor expressed in melanocytes[35] and induces Tyr expression[36]. In our experiment, Tyr expression was slightly suppressed without significance. Thus, fucoxanthin was found to mildly affect EDNRA expression leading to Tyr expression. Fucoxanthin significantly suppressed expression of p75NTR, but not NT3R. NT-3 receptors are divided into two types.[37] p75NTR is a receptor with low affinity to NT-3. A low concentration of fucoxanthin (0.01 and 0.1%) significantly suppressed mRNA expression of p75NTR. The same result was obtained from an experiment using oral treatment with fucoxanthin. Namely, fucoxanthin (10 mg/kg) suppressed the expression of p75NTR but not NT-3. These results suggest that fucoxanthin selectively suppresses expression of low affinity NT-3 receptors by topical and oral treatment. EP1 is a PGE_2 receptor expressed in melanocytes and it enhances melanocyte differentiation.[38] UVB mainly induces PGE_2 production by COX-2.[39] As topical (1%) and oral (10 mg/kg) treatment with fucoxanthin significantly suppressed EP1 expression, fucoxanthin was found to affect both epidermal cells and melanocytes. Suppression of PG synthesis and its receptor expression seems to be involved in the inhibitory mechanism on melanogenesis exhibited by fucoxanthin. MC1R is a receptor of MSH and plays a critical role in skin pigmentation. It is regarded as a target molecule for development of new topical medicines.[40] In naturally occurring anti-pigmentary compounds, acteoside, a polyphenolic compound, was reported to suppress cAMP elevation via binding of MSH to MC1R.[41] Topical (0.01 to 1%) and oral (10 mg/kg) treatment of fucoxanthin significantly suppressed MC1R expression. We evaluated POMC

expression, which is a precursor of MSH, but the expression was not observed in this assay system. Hence, fucoxanthin seems to mainly suppress expression of the MSH receptor in POMC-MSH system.

In mRNA expression of Tyr and Tyrp1 related to melanogenesis in melanocytes, topical treatment with fucoxanthin (0.1 and 1%) significantly suppressed Tyrp1 expression. The expression of Tyr was slightly suppressed by the same concentration of fucoxanthin without significance. Thus, fucoxanthin was found to mainly suppress Tyrp1 in the melanin production system. We summarized the effects of fucoxanthin on melanogenesis in Figure 4. Comparing molecules affected by fucoxanthin in the cells distributed in skin, fucoxanthin is considered to act mainly on melanogenic molecules in melanocytes. Especially, p75NTR, EP1 and MC1R are strongly suppressed by topical treatment with fucoxanthin. Suppression of the expression of these molecules may indirectly suppress Tyrp1 expression. Moreover, topical treatment with fucoxanthin suppressed the molecules more strongly as compared with oral treatment. These results suggest that the use of fucoxanthin may provide a new anti-pigmentary tool in medicinal and cosmetic fields.

Conclusion

Fucoxanthin suppressed tyrosinase activity, melanogenesis in melanoma and pigmentation in UV-irradiated guinea-pigs. In the melanogenesis pathway, fucoxanthin inhibited mRNA expression of cyclooxygenase-2, endothelin receptor A, p75 neurotrophin receptor, prostaglandin E receptor and melanocortin 1 receptor following topical and oral application. In addition to the tyrosinase inhibitory activity, fucoxanthin is suggested to exhibit anti-melanogenic activity via suppression of prostaglandin synthesis and expression of melanogenic receptors in melanocytes.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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