Journal of Cellular Biochemistry, Supplement 22:231–235 (1995)

Cancer Chemoprevention by Natural Carotenoids and Their Related Compounds

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Abstract As one of the most promising cancer chemopreventive agents, β-carotene has been studied extensively. However, other natural carotenoids have also suppressed tumorigenesis, and some are more potent than β-carotene. For example, α-carotene shows higher potency than β-carotene in suppressing tumorigenesis in mouse skin and lung models. In the two-stage mouse skin carcinogenesis model (initiator, 7,12-dimethylbenz[*a*]anthracene; promoter, 12-*O*-tetradecanoylphorbol-13-acetate), topical application of α-carotene at a 200 nmol dose per painting twice a week significantly decreased the mean number of skin tumors per mouse. The greater potency of α-carotene over β-carotene in suppression of tumor promotion was confirmed in the two-stage mouse lung carcinogenesis model (initiator, 4-nitroquinoline-1-oxide; promoter, glycerol). Oral administration of α-carotene (0.05% in drinking water) significantly decreased the mean number of lung tumors per mouse. In contrast, β-carotene showed no suppression of lung tumor formation under the same experimental conditions. Fucoxanthin, a carotenoid as abundant in nature as β-carotene, was also found to have antitumorigenic activity in mouse skin and duodenum models. Thus, further studies on various natural carotenoids, other than β-carotene, should be carried out in the field of cancer chemoprevention.

Key words: Cancer chemoprevention, α-carotene, carotenoids, fucoxanthin

Epidemiological investigations have shown that cancer risk is inversely related to the consumption of green and yellow vegetables [1–2]. Since β-carotene is present in abundance in these vegetables, it has been proposed as an important factor for cancer prevention. However, it was recently reported that supplements of β-carotene increased the incidence of lung cancer among heavy smokers in Finland [3]. One possible explanation is that in epidemiologic studies, β-carotene merely acts as a marker for cancer chemopreventive agents which co-exist with β-carotene in green and yellow vegetables. In fact, various kinds of cancer preventive substances have been identified in green and yellow vegetables. In the case of carotenoids, β-carotene is usually associated with other natural carotenoids, such as α-carotene, lutein, zeaxanthin, lycopene and β-cryptoxanthin, and these carotenoids are also detectable in human serum and tissues. In this context, we evaluated the cancer chemopreventive potency of natural carotenoids other than β-carotene.

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MATERIALS AND METHODS

Palm carotene was prepared by a previously reported method [4]. α-Carotene was purified from palm carotene by high-performance liquid chromatography with a lime-packed column. β-Carotene was purchased from Sigma. These carotenes were prepared as emulsions as described previously [5]. Other carotenoids, which were supplied by Dr. Tanaka or Dr. Tsushima, were dissolved in dimethyl sulfoxide, and then

dispersed into water or medium. Female ICR mice, male ddY mice, male C3H/He mice, and male C57Bl/6 mice were used as model systems for carcinogenesis experiments in skin, lung, liver, and duodenum, respectively (Table I).

RESULTS

Effect of Palm Carotene on Carcinogenesis of Skin, Lung, Liver, and Duodenum

Palm carotene showed potent antitumor promoting activity in a two-stage carcinogenesis experiment in skin, initiated with dimethylbenz[*a*]anthracene (DMBA) and promoted with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). In the control group, the first tumor appeared within 6 weeks of promotion; at the end of the experiment, 96.7% of mice developed skin tumors, with an average number of 2.63 per mouse. On the contrary, palm carotene treatment resulted in the complete suppression of tumor formation, *i.e.*, no skin tumors developed during the whole period of the experiment. The antitumor promoting activity of palm carotene was confirmed by another two-stage carcino-

TABLE I. Antitumorigenic Activity of Palm Carotene

Group	(n)	Percentage of Tumor-Bearing Mice	Mean Tumors/Mouse
Skin Carcinogenesis[1]			
Control	(30)	96.7	2.63
+ Palm carotene	(10)	00.0	0.00
Lung Carcinogenesis[2]			
Control	(14)	100.0[a]	3.06[b]
+ Palm carotene	(12)	33.3[a]	0.58[b]
Liver Carcinogenesis[3]			
Control	(16)	100.0	6.31[c]
+ Palm carotene	(15)	100.0	3.60[c]
Duodenal Carcinogenesis[4]			
Control	(27)	66.7[d]	0.93
+ Palm carotene	(28)	39.3[d]	0.64

[a] $p < 0.05$, [b] $p < 0.001$, [c] $p < 0.01$, [d] $p < 0.05$

[1] Tumor initiation was accomplished by a single application of DMBA (100 μg) on the shaved backs of mice. The tumor promotor TPA was applied at a dose of 0.81 nmol/painting twice a week starting 1 week after initiation. Palm carotene (162 nmol) in 200 μl of acetone was applied simultaneously with each application of TPA. Controls were treated with the same amount of vehicle for palm carotene. The experiment continued up week 16 of promotion.

[2] The tumor initiator, 4NQO (10 μg/kg body weight), dissolved in a mixture of olive oil and cholesterol (20:1) was given by a single sc injection on the first experimental day. Glycerol (tumor promoter) was dissolved in drinking water at a concentration of 10%, and given *ad libitum* during experimental weeks 5–30. Palm carotene (at a concentration of 0.005%) or vehicle control was mixed as an emulsion into drinking water during tumor promotion.

[3] Mice received palm carotene (at 0.005% concentration) or vehicle as an emulsion in drinking water for 40 weeks.

[4] ENNG (0.01%) in drinking water was given *ad libitum* for the first 4 weeks. Then, palm carotene (0.05%) or vehicle dissolved as an emulsion in drinking water was given *ad libitum* for 16 weeks.

TABLE II. Effects of α- and β-Carotene on Tumorigenesis in Skin and Lung

Group	(n)	Percentage of Tumor-Bearing Mice	Mean Tumors/Mouse
Skin Carcinogenesis[1]			
Control	(16)	68.8	3.73[a]
+ α-Carotene	(16)	25.0	0.27[a]
+ β-Carotene	(16)	31.3	2.94
Lung Carcinogenesis[2]			
Control	(16)	93.8	4.06[b]
+ α-Carotene	(15)	73.3	1.33[b]
+ β-Carotene	(15)	93.3	4.93

[a]$p < 0.01$, [b]$p < 0.001$

1 Two-stage carcinogenesis experiments were carried out as described in Table I, with modifications. Doses of TPA and carotenes were 1.62 nmol and 200 nmol, respectively. The experiment was continued for 20 weeks.

2 Two-stage carcinogenesis experiments were carried out as described in Table I, with modifications. The concentrations were 0.05%.

genesis experiment. We examined the effect of palm carotene on the promotion of lung tumor formation in 4-nitroquinoline-1-oxide (4NQO)-initiated mice. Oral administration of palm carotene (at a dose of 0.005% in drinking water, *ad libitum*) decreased the mean number of tumors per mouse to about 19% of the number in the control group ($p < 0.001$). Palm carotene also significantly decreased the percentage of tumor-bearing mice ($p < 0.05$). In spontaneous liver carcinogenesis in C3H/He male mice, the mean number of hepatomas was significantly decreased by oral administration of palm carotene (at a dose of 0.005% in drinking water, *ad libitum*) as compared with the control group; the control group developed 6.31 tumors/mouse, whereas the palm carotene-treated group had 3.60 tumors/mouse ($p < 0.01$). Palm carotene also suppressed *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine (ENNG)-induced duodenal tumorigenesis. The percentage of tumor-bearing mice was significantly decreased by oral administration of palm carotene (at a dose of 0.05% in drinking water, *ad libitum*) as compared with that in the control group; 66.7% of mice in the control group developed tumors, whereas 39.3% of mice developed tumors in the palm carotene-treated group ($p < 0.05$).

Effect of α- and β-Carotene on Skin and Lung Carcinogenesis

Since palm carotene, which consists of 60% β-carotene, 30% α-carotene and 10% others (γ-carotene, lycopene, *etc.*) showed significant antitumorigenic effects, we further examined the effect of α-carotene, one of the major constituents, compared to β-carotene. As shown in Table II, α-carotene was more potent than β-carotene in suppressing tumorigenesis in skin and lung. In two-stage skin carcinogenesis, α-carotene significantly decreased the mean number of tumors per mouse to about 7% of the control group ($p < 0.01$). β-Carotene treatment also decreased the mean number of tumors per mouse, but the difference from the control group was not significant. The percentage of tumor-bearing mice in the control group was 68.8%, whereas the percentages in the groups treated with α- or β-carotene were 25.0% and 31.3%, respectively. Thus, α-carotene was more inhibitory than β-carotene, though both α- and β-carotene inhibited

TABLE III. Antitumorigenic Activity of Fucoxanthin

Group	(n)	Percentage of Tumor-Bearing Mice	Mean Tumors/Mouse
Skin Carcinogenesis[1]			
Control	(15)	53.3	2.20
+ Fucoxanthin	(15)	00.0	0.00
Duodenal Carcinogenesis[2]			
Control	(18)	77.8[a]	1.28[b]
+ Fucoxanthin	(20)	30.0[a]	0.55[b]

[a]$p < 0.005$, [b]$p < 0.05$

[1] Two-stage carcinogenesis experiments were carried out as described in Table I, with modifications. Doses of TPA and fucoxanthin were 1.62 nmol and 0.6 μmol, respectively. The experiment was continued for 20 weeks.

[2] Experiment was carried out as described in Table I, with some modifications. The concentration of fucoxanthin was 0.005%.

skin tumor formation promoted by TPA. The greater potency of α-carotene in suppressing tumor promotion was confirmed by another two-stage carcinogenesis experiment. We examined the effects of α- and β-carotene on the promotion of lung tumor formation in 4NQO-initiated mice. Oral administration of α-carotene significantly decreased the mean number of tumors per mouse; the control group developed 4.06 tumors/mouse, whereas the α-carotene-treated group had 1.33 tumors/mouse ($p < 0.001$). α-Carotene also showed a tendency to decrease the percentage of tumor-bearing mice, although the difference was not statistically significant. On the contrary, β-carotene showed no suppression under the same experimental conditions as α-carotene.

Effect of Fucoxanthin on Carcinogenesis of Skin and Duodenum

These results indicate that we should pay more attention to antitumorigenic activities of natural carotenoids other than β-carotene. In this context, we examined the effect of fucoxanthin on tumorigenesis in skin and duodenum. Fucoxanthin, as well as β-carotene, is one of the most abundant carotenoids in nature; it is especially widely distributed in marine organisms, including seaweeds. Fucoxanthin used in this experiment was prepared from the brown algae *Hijikia fusiforme*, a common edible seaweed in Japan. As shown in Table III, fucoxanthin (at a dose of 0.6 μmol per painting) completely suppressed skin tumor formation during the entire experiment—up to 20 weeks of promotion. Fucoxanthin also suppressed ENNG-induced mouse duodenal carcinogenesis. The percentage of tumor-bearing mice in the control group and in the fucoxanthin-treated group was 77.8% and 30.0%, respectively, a statistically significant difference ($p < 0.005$). The mean number of tumors per mouse was also significantly decreased by fucoxanthin; control mice developed 1.28 tumors/mouse, whereas fucoxanthin-treated mice had 0.55 tumors/mouse ($p < 0.05$).

DISCUSSION

Since α-carotene and fucoxanthin were proven to suppress tumorigenesis, we expanded the study to test the anticarcinogenic activity of various natural carotenoids and their related compounds. For example, lutein and peridinin were found to have antitumor promoting activity in mouse skin carcinogenesis. It is of interest to examine whether lutein and peridinin also suppress carcinogenesis in organs other than skin. Peridinin has a butenolide ring in its structure, which may have specific biological activity.

Therefore, we synthesized various kinds of butenolide compounds and evaluated their antitumorigenic activity. Some of them, such as (1E, 3E,5E,7E)-5-hydroxy-4-(8-phenyl-1,3,5,7-octatetraenyl)-2(5H)-furanone, proved to have cancer preventive activity.

In conclusion, various kinds of natural carotenoids and related compounds other than β-carotene seem to be promising chemopreventive agents. Thus, further studies on these agents should be carried out.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Health and Welfare, the Ministry of Education, Science and Culture, the Smoking Research Foundation, and the Plant Science Research Foundation, Faculty of Agriculture, Kyoto University, Japan. The work was carried out in collaboration with research groups of the Kyoto Prefectural University of Medicine, Kagoshima University, Kyoto Pharmaceutical University, Kobe Women's College of Pharmacy, Lion Co., and Kuraray Co., Japan.

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