Carotenoids Affect Proliferation of Human Prostate Cancer Cells[1]

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ABSTRACT We investigated whether various carotenoids present in foodstuffs were potentially involved in cancer-preventing action on human prostate cancer. The effects of 15 kinds of carotenoids on the viability of three lines of human prostate cancer cells, PC-3, DU 145 and LNCaP, were evaluated. When the prostate cancer cells were cultured in a carotenoid-supplemented medium for 72 h at 20 μmol/L, 5,6-monoepoxy carotenoids, namely, neoxanthin from spinach and fucoxanthin from brown algae, significantly reduced cell viability to 10.9 and 14.9% for PC-3, 15.0 and 5.0% for DU 145, and nearly zero and 9.8% for LNCaP, respectively. Acyclic carotenoids such as phytofluene, ζ-carotene and lycopene, all of which are present in tomato, also significantly reduced cell viability. On the other hand, phytoene, canthaxanthin, β-cryptoxanthin and zeaxanthin did not affect the growth of the prostate cancer cells. DNA fragmentation of nuclei in neoxanthin- and fucoxanthin-treated cells was detected by in situ TdT-mediated dUTP nick end labeling (TUNEL) assay. Neoxanthin and fucoxanthin were found to reduce cell viability through apoptosis induction in the human prostate cancer cells. These results suggest that ingestion of leafy green vegetables and edible brown algae rich in neoxanthin and fucoxanthin might have the potential to reduce the risk of prostate cancer. J. Nutr. 131: 3303–3306, 2001.

KEY WORDS: • neoxanthin • fucoxanthin • carotenoid • prostate cancer cell • cancer prevention

Prostate cancer has become the second leading cause of cancer-related death among men in most Western countries (1). Epidemiologic studies suggest that consumption of vegetables and fruits reduces the risk of prostate cancer (2–4). Giovannucci et al. (2) reported that ingestion of tomato-based foods and increased plasma lycopene level (5) were significantly associated with a lower risk of prostate cancer. Cook et al. (4) suggested that β-carotene supplementation might reduce the risk of prostate carcinoma among men with low baseline levels of plasma β-carotene. The chemopreventive effects of carotenoids on prostate cancer have also been indicated in several studies with cell lines. Williams et al. (6) reported that β-carotene at a concentration of >30 μmol/L significantly reduced the growth of three human prostate cancer cell lines, PC-3, DU 145 and LNCaP. Pastori et al. (7) reported that lycopene at 1 μmol/L in association with 50 μmol/L α-tocopherol inhibited the proliferation of PC-3 and DU 145 cells, whereas lycopene alone was not a potent inhibitor of prostate cancer cell proliferation. Moreover, carotenoids have been found to inhibit the growth of several cancer cell lines in addition to prostate cancer cells, including melanoma (8), lung (9), mammary (9), colon (10) and leukemia cancer cells (11). Furthermore, carotenoid treatment has been reported to cause cell-cycle arrest (12), enhancement of gap junctional communications (13), inhibition of the malignant transformation in C3H/10T1/2 cells (14), and induction of apoptosis (15–18) and differentiation (11). Together, these studies indicate that carotenoids present in vegetables and fruits may be responsible for potential cancer-preventing action by inhibiting the growth of tumor cells.

β-Carotene and lycopene are the major dietary carotenoids, but other carotenes and several xanthophylls are also present in substantial amounts in edible plants. Tomatoes contain acyclic carotenoids such as phytoene, phytofluene and ζ-carotene as well as lycopene (19,20), and large amounts of these acyclic carotenoids accumulate in human serum (21). Indeed, we recently found that phytofluene and ζ-carotene inhibited cell growth by inducing apoptosis in HL-60 cells (18). However, the effects of carotenoids other than β-carotene and lycopene on the growth of prostate cancer cells are unknown. In the present study, we evaluated the effects of 15 kinds of carotenoids present in foodstuffs on the growth of three human prostate cancer cell lines, PC-3, DU 145 and LNCaP, to investigate the possible cancer-preventing action of carotenoids against human prostate cancer.

MATERIALS AND METHODS

Materials. Tomato oleoresin (Lyc-O-Mato 6%) was kindly donated by Ajinomoto Takara (Tokyo, Japan). All-*trans*-α-carotene, all-*trans*-β-carotene (Type III), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)[3] were purchased from Sigma Chemical (St. Louis, MO). β-Cryptoxanthin and zeaxanthin were purchased from Extrasynthese SA (Genay, France). Astaxanthin and canthaxanthin were kindly donated by Nippon Roche (Tokyo, Japan). Capsanthin was kindly donated by Kagome (Tokyo, Japan). Lutein was kindly donated by Kyowa Hakko Kogyo (Tokyo, Japan). Brown algae (*Undaria pinnatifida*) and spinach (*Spinacia oleracea L.*)

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[3] Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; THF, tetrahydrofuran; TUNEL, TdT-mediated dUTP nick end labeling.

were purchased from a local market in Tsukuba, Japan. Apoptosis in situ Detection Kit Wako was purchased from Wako Pure Chemical (Osaka, Japan). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from CSL Limited (Victoria, Australia). HPLC-grade tetrahydrofuran (THF) and acetonitrile were purchased from Nacalai Tesque (Kyoto, Japan). Other chemicals and solvents were of reagent grade.

Preparation of carotenoids. Phytoene, phytofluene, ζ-carotene and lycopene were isolated from tomato oleoresin as previously reported (18). Fucoxanthin was isolated from brown algae, and neoxanthin and violaxanthin were isolated from spinach leaf, using a method published previously (22). α-Carotene, β-carotene, lutein, β-cryptoxanthin and canthaxanthin were purified with a neutral alumina III column. Astaxanthin and capsanthin were purified with a silica gel column. The carotenoids were stored at −80°C. The purity of all carotenoids was > 99%, based on the peak area of all components absorbing at each specific wavelength in HPLC analysis. The extinction coefficient of each carotenoids (E1%, 1 cm) was used for quantification (23).

Cell culture and MTT assay. PC-3, DU 145 and LNCaP human prostate cancer cells were obtained from the American Type Culture Collection (Rockville, MD). These cell lines were cultured in DMEM supplemented with 10% heat-inactivated FBS, 4 mmol/L L-glutamine and antibiotics (40 g/L penicillin and 40,000 U/L streptomycin) at 37°C in a humidified atmosphere of 5% CO_2 in air. These cell lines were passed twice a week. To evaluate the effect of the carotenoids on the viability of PC-3, DU 145 and LNCaP cells, the cells were seeded at a density of 5×10^3 cells per well containing 100 μL of culture medium in 96-well plates. After 24 h of cultivation, the medium was changed to fresh medium supplemented with a carotenoid. The carotenoids, dissolved in distilled THF, were added to the culture medium at a final concentration of 5, 10 or 20 μmol/L. The final concentration of THF in the culture medium was 0.5% (v/v), and the control culture received only THF (vehicle alone). After 72 h of cultivation, cell viability was evaluated by MTT assay (24) and was expressed as the percentage of the value of the control culture treated with the vehicle alone (THF).

Statistical analysis. Data represent the means ± SD. Results were analyzed by one-way ANOVA and Scheffé's *F*-test to identify significant differences between groups. *P*-values < 0.01 were considered significant. All analyses were performed using StatView software version 4.5J (Abacus Concepts, Berkeley, CA).

TdT-mediated dUTP nick end labeling assay. Cells were seeded in 24-well plates at a density of 2×10^4 cells/well and treated with a carotenoid as described above for the MTT assay. After 24 h of cultivation, the cells were fixed using 4% paraformaldehyde for 10 min at room temperature. DNA fragmentation was detected by TdT-mediated dUTP nick end labeling (TUNEL) (25) using an Apoptosis in situ Detection Kit Wako. The DNA fragmentation appeared as red-stained nuclei. Cells were visualized using a microscope.

All experiments were done under dim yellow light to minimize isomerization and degradation of carotenoids by light irradiation.

RESULTS

Effects of the carotenoids on the viability of human prostate cancer cells. At 20 μmol/L, acyclic carotenoids such as phytofluene, ζ-carotene and lycopene significantly reduced cell viability to 60.6, 63.6 and 58.7% for PC-3 and to 67.3, 71.9 and 54.1% for DU 145 of the control culture with vehicle alone, respectively. However, these acyclic carotenoids, except for 20 μmol/L phytofluene, did not reduce the cell viability of LNCaP (**Fig. 1**). At a concentration of 5 μmol/L, lycopene generally reduced cell viability more than did the other acyclic carotenoids, α-carotene and β-carotene. In particular, the reduction of cell viability of DU 145 by lycopene was significantly greater than those by the other carotenoids described above ($P < 0.01$). α-Carotene and β-carotene at 20 μmol/L significantly reduced the viability of the three prostate cancer cells, except for β-carotene vs. LNCaP. Astaxanthin at 20 μmol/L significantly reduced the cell viability of PC-3, but did not reduce the cell viability of either DU 145 or LNCaP. Lutein at 20 μmol/L significantly reduced the viability of PC-3 and DU 145 cells. Violaxanthin and capsanthin significantly reduced the viability of PC-3 and DU 145 cells, but the cell viability was independent of the carotenoid concentration. On the other hand, phytoene, canthaxanthin, β-cryptoxanthin and zeaxanthin did not reduce the viability of human prostate cancer cells under the conditions tested. Among the carotenoids examined, neoxanthin and fucoxanthin were the most effective compounds in reducing cell viability. The percentages of viable cells in the media supplemented with neoxanthin and fucoxanthin at 20 μmol/L were 10.9 and 14.9% for PC-3, 15.0 and 5.0% for DU 145, and nearly zero and 9.8% for LNCaP, respectively. The cell viability depended on the carotenoid concentration. Furthermore, these two carotenoids at 20 μmol/L reduced significantly the viability of the three prostate cancer cells more than did the other carotenoids tested ($P < 0.01$), except for neoxanthin vs. violaxanthin, α-carotene or phytofluene in LNCaP.

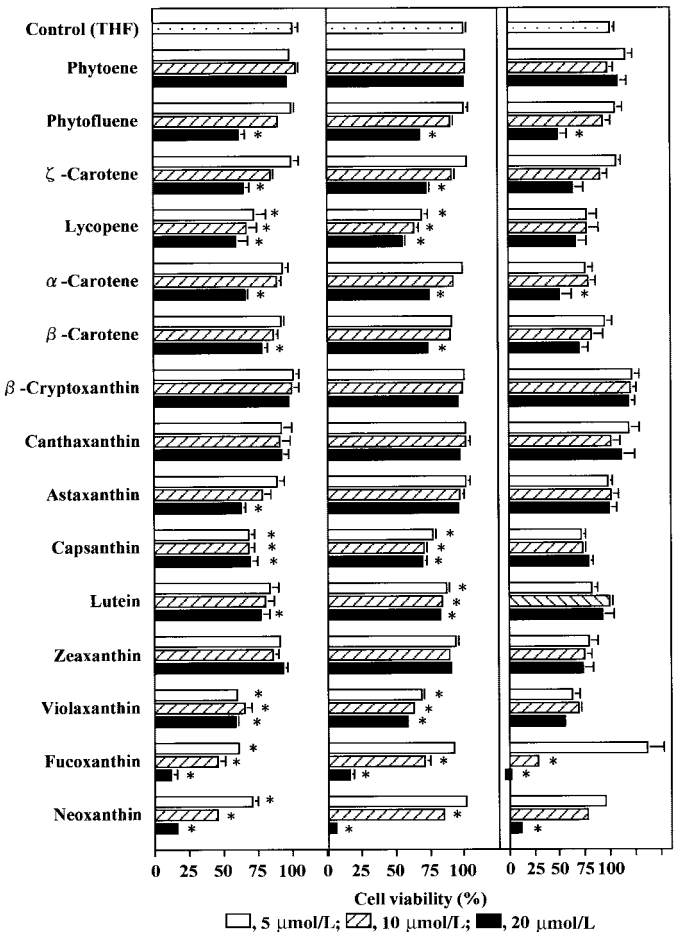

FIGURE 1 Effects of 15 carotenoids on the viability of human prostate cancer cells. PC-3 (*left panel*), DU 145 (*center panel*) and LNCaP (*right panel*) cells were seeded in 96-well plates and cultured for 24 h, and then treated with various carotenoids. After culturing for 72 h, cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and was expressed as the percentage of the value of the control culture treated with the vehicle alone (tetrahydrofuran). The values represent means ± SD of eight wells. Replicate experiments demonstrated a similar trend. The asterisk indicates a value significantly different from the vehicle control value ($P < 0.01$). Statistical comparisons were made by Scheffé's *F*-test.

TUNEL assay. Exposure of the neoxanthin and fucoxanthin at 20 μmol/L to the three human prostate cancer cells clearly induced the morphological changes characterized by rounding up, detachment, reduction of cell volume and apoptotic bodies (data not shown). We examined whether neoxanthin and fucoxanthin could induce apoptosis in the prostate cancer cells. The nuclei with DNA fragmentation, red-stained by TUNEL assay, were observed in the three prostate cancer cells treated by neoxanthin and fucoxanthin at 20 μmol/L, and typical pictures of PC-3 cells are shown in **Figure 2**. These results indicated that neoxanthin and fucoxanthin induced apoptosis in the PC-3, DU 145 and LNCaP cell lines.

DISCUSSION

We evaluated the effects of 15 carotenoids present in foodstuffs on the growth of the human prostate cancer cell lines, PC-3, DU 145 and LNCaP. Lycopene, at a low concentration (5 μmol/L), tended to reduce the cell viability more than did the other acyclic carotenoids, α-carotene and β-carotene. These findings suggest that lycopene has a higher potential for cancer-preventive action than do the other carotenoids. However, a recent study in which lycopene was fed to rats did not show the repressed development of chemically induced prostate carcinoma (26), although the bioavailability of carotenoids in rats is much lower than that in humans. Further studies using appropriate animal models will be required to evaluate the possible cancer-preventing action of lycopene in vivo. On the other hand, acyclic carotenoids such as phytofluene and ζ-carotene at a higher concentration (20 μmol/L) reduced cell viability as strongly as did lycopene in the present study. Phytofluene and ζ-carotene are ingested together with lycopene and accumulate in human serum in substantial amounts. The combined amount of phytofluene and ζ-carotene accumulated in human serum was comparable to that of β-carotene (21). The present study is the first to demonstrate that phytofluene and ζ-carotene as well as lycopene reduce the viability of human prostate cancer cells and suggest their involvement in preventing prostate cancer.

Williams et al. (6) reported that β-carotene at >30 μmol/L significantly inhibited the growth of PC-3, DU 145 and LNCaP cells. In the present study, α-carotene and β-carotene at 20 μmol/L significantly reduced the viability of those cells. However, β-cryptoxanthin did not reduce cell viability, and most of the nonprovitamin A carotenoids at 20 μmol/L reduced the cell viability of PC-3. These results suggest that the provitamin A activity is not associated with the inhibitory effects on the viability of prostate cancer cells.

Neoxanthin and fucoxanthin at >10 μmol/L each showed a remarkable reduction in the viability of prostate cancer cells in the present study. Furthermore, DNA fragmentation, indicated by in situ TUNEL, suggests that these two carotenoids apparently reduce cell viability by inducing apoptosis. This is the first study to show apoptosis induction by neoxanthin and fucoxanthin in human prostate cancer cells. Chang and Lin (27) reported that neoxanthin strongly inhibited cell growth by suppressing DNA synthesis in C3H10T1/2 cells, and that neoxanthin inhibited 7,12-dimethylbenz [a] anthracene–induced carcinogenesis in hamster buccal pouch (28). Fucoxanthin has been shown to inhibit the expression of N-myc oncogene, cell cycle progression in the human neuroblastoma cell line, GOTO cells (29) and *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine–induced mouse duodenal carcinogenesis (30). The apoptosis induction by fucoxanthin has also been observed in HL-60 cells (31). Halocynthiaxanthin, a metabolite of fucoxanthin, has been reported to inhibit the growth of GOTO cells (32). These three carotenoids each have a 5,6-monoepoxide in the molecule, suggesting that this chemical structure is associated with the biological effects of these carotenoids. Duitsman et al. (33) reported that 5,6-monoepoxide of β-carotene showed greater activity of inducing differentiation in NB4 human leukemia cells than did the 5,8-monoepoxide, the 5,6,5′,6′-diepoxide or β-carotene. Similar results were obtained in the present study. Violaxanthin with a 5,6,5′,6′-diepoxide showed lower activity in the reduction of viability of the prostate cancer cells than did neoxanthin or fucoxanthin with the 5,6-monoepoxide at 20 μmol/L. Furthermore, an allenic bond present in neoxanthin and fucoxanthin may also be involved in the reduction of cell viability. Antioxidant actions of neoxanthin and fucoxanthin were shown in several studies (27,34). However, carotenoids act not only as antioxidants but also as prooxidants, and the prooxidant action of carotenoids has been suggested to induce apoptosis in tumor cells (16). Neoxanthin and fucoxanthin with conjugated double bonds and 5,6-monoepoxide are thought to be highly susceptible to acids, alkalis and oxygen. Their prooxidant actions might cause apoptosis induction in the prostate cancer cells.

Neoxanthin is ingested via leafy green vegetables in most diets because it is one of the major carotenoids present in the chloroplast of higher plants. People in East Asian countries, in which the mortality rate from prostate cancer is low, (35) ingest fucoxanthin through edible brown algae such as *Undaria pinnatifida*, *Laminaria japonica* and *Hizikia fusiformis*. Thus, consumption of leafy green vegetables and brown algae might

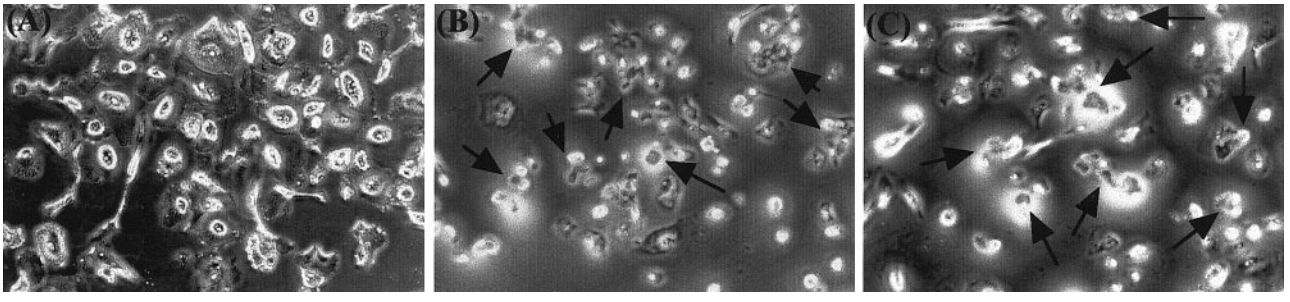

FIGURE 2 Effects of neoxanthin and fucoxanthin on DNA fragmentation in human prostate cancer cells. PC-3 cells were seeded and cultured for 24 h, and then treated with (*A*) tetrahydrofuran (THF) alone, (*B*) 20 μmol/L neoxanthin and (*C*) 20 μmol/L fucoxanthin. After culturing for 24 h, cells were fixed using 4% paraformaldehyde, and nuclei with DNA fragmentation were examined with an Apoptosis in situ Detection Kit Wako by the TdT-mediated dUTP nick end labeling (TUNEL) method. DNA fragmentation was demonstrated by red staining of nuclei compared with cells counterstained green. Typical apoptotic cells in (*B*) and (*C*) are indicated by arrows. The cells were photographed with a microscope (X200).

reduce the risk of prostate cancer. However, it is still unresolved whether neoxanthin and fucoxanthin can be absorbed and accumulated in the human body. The stability of these carotenoids in the digestive organs also is unknown. The mechanism of apoptosis induction by neoxanthin and fucoxanthin on prostate cancer cells and their potential for cancer prevention in experimental animals and human subjects deserve further study.

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