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Antioxidant and pro-oxidant effects of lycopene in comparison with β-carotene on oxidant-induced damage in Hs68 cells

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Lycopene has become a focal point in recent research following clinical trials that suggest that β-carotene may promote lung cancer in smokers. Because lycopene only differs from β-carotene in lacking the β-ionone structure, and β-carotene is known to have pro-oxidant activity in vitro, we sought to determine whether lycopene might also have pro-oxidant activity in vitro in comparison with β-carotene. Human foreskin fibroblasts (Hs68 cells) were first enriched with 10 and 20 μM lycopene or β-carotene for 1 hr followed by incubation with various oxidants. Lipid peroxidation was measured as thiobarbituric acid-reactive substances (TBARS) released into the medium and DNA damage was measured as formation of comet and 8-hydroxy-2′-deoxyguanosine. The results showed that lycopene at 20 μM significantly decreased levels of TBARS induced by ferric nitrilotriacetate (Fe/NTA) but enhanced levels of TBARS induced by a lipid-soluble radical generator (2,2′-azobis[2,4-dimethylvaleronitrile]; AMVN). Both the antioxidant and pro-oxidant effects of lycopene tended to be dose-dependent. β-Carotene at 20 μM did not significantly decrease TBARS induced by Fe/NTA but significantly increased TBARS induced by AMVN. Lipid peroxidation induced by a water-soluble radical generator 2,2'-azobis(2-amidinopropane)dihydrochloride was not significantly affected by either lycopene or β-carotene. Neither lycopene nor β-carotene affected DNA damage or changes in cell morphology induced by any of the three oxidants tested. The present study in Hs68 cells demonstrates that lycopene can be either an antioxidant or a pro-oxidant depending on the oxidants used, and that lycopene and β-carotene behave similarly under the in vitro oxidative conditions. Although it is unclear whether lycopene may have pro-oxidant activity in vivo, our results caution that it may be premature to undertake clinical trials with lycopene. (J. Nutr. Biochem. 11:548–554, 2000)

Keywords: lycopene; β-carotene; lipid peroxidation; DNA damage; cell culture

Introduction

Carotenoids are a class of more than 600 natural pigments that are present in fruits and vegetables.[1] Several carotenoids, especially β-carotene, have received tremendous research interest because they are found in high concentration in human blood.[2] Epidemiological studies have shown an inverse relationship between the intake of fruits and vegetables and the risk of several types of cancer,[3] and such effects of fruits and vegetables have been attributed to β-carotene.[4] Both in vitro and animal studies have produced supportive results to this theory. Most notably, β-carotene is shown to be an effective antioxidant by scavenging certain reactive oxygen species (ROS), especially peroxyl radical and singlet oxygen, and this antioxidant activity appears to be greatest at low oxygen tension.[5,6]

However, recent clinical trials of supplemental β-carotene on the incidence and mortality of cancer and cardiovascular disease have produced disappointing results, that is, β-carotene supplementation is either nonprotective[7] or even detrimental in smokers.[8,9] Thus, the antioxidant and anticarcinogenic properties of β-carotene remain obscure and must be reexamined closely. A recent study in normal and tumor thymocytes showed that β-carotene is an antiox-

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idant at low oxygen pressure but a pro-oxidant at high oxygen concentrations.[10] In fact, the dependence of β-carotene's antioxidant (or pro-oxidant) activity on oxygen pressure was noted earlier.[5] β-Carotene was also shown to enhance lipid peroxidation induced by NO_2.[11] A few studies that have investigated effects of β-carotene on DNA damage have produced conflicting results. For instance, a study in six nonsmoking and six smoking volunteers showed that a single dose of 45 mg β-carotene lowers oxidative damage to lymphocytic DNA determined by the comet assay.[12] By contrast, serum β-carotene levels were not found to be associated with the degree of DNA damage measured by the comet assay.[13] It has recently been suggested that some of the degradation products of β-carotene, rather than β-carotene itself, may be pro-oxidant or procarcinogenic.[14] For example, in an experiment involving incubation of calf thymus DNA with rat liver microsomes treated with cytochrome P450-inducing compounds, it was shown that some of the degradation products of β-carotene enhance the binding of benzo[a]pyrine to DNA, although β-carotene itself inhibits the binding.[15] This observation may help explain the finding that β-carotene enhances respiratory tract cancer induced by benzo[a]pyrene in hamsters.[16]

Recently, lycopene, a acyclic non-provitamin A carotenoid with 11 linearly arranged conjugated double bounds and found in relatively few foods, has received much research attention following the disappointing results from β-carotene clinical trials because of its potential antioxidant and antiproliferative properties.[1] Lycopene has somewhat higher antioxidant activity than β-carotene. For example, lycopene is destroyed to a greater extent than β-carotene in human skin irradiated with UV.[17] Other studies have shown that lycopene is more efficient than β-carotene in scavenging singlet oxygen[18] and peroxyl radicals[19] and in protecting lymphocytes against NO_2-induced membrane damage and cell death.[20,21] However, contradictory findings also exist. For instance, when isolated low density lipoprotein (LDL) was enriched with various carotenoids in vitro, β-carotene but not lycopene (and lutin) protected against oxidation by copper ions, despite the fact that lycopene was destroyed faster than β-carotene.[22] Similarly, in liposomes oxidized by peroxyl radicals, exogenous lycopene was destroyed more rapidly than other carotenoids including β-carotene, but was least effective as an antioxidant.[23] In addition, a study in healthy humans showed that dietary supplementation with β-carotene, but not lycopene (in the form of tomato juice), inhibits endothelial cell-mediated LDL oxidation.[24] Interestingly, in this human study, one of the three subjects with a higher serum lycopene level had elevated LDL oxidation.[24] It is important to note that lycopene is only structurally different from β-carotene in lacking the β-ionone ring (and thus devoid of vitamin A activity). Thus, the possibility that lycopene may have similar pro-oxidant activities as has β-carotene cannot be ignored. As very little work on lycopene has been done using cell culture systems, the present study employed human foreskin fibroblasts (Hs68 cells) to investigate the antioxidant and pro-oxidant activities of lycopene in comparison with β-carotene under oxidative stress. The oxidants used included a lipid-soluble radical generator (2,2′-azobis[2,4-dimethylvaleronitrile]; AMVN), a water-soluble radical generator (2,2′-azobis[2-amidinopropane]dihydrochloride; AAPH), and ferric nitrilotriacetate (Fe/NTA), a hepatic and renal carcinogen known to induce both lipid peroxidation and DNA damage.[25,26]

Material and methods

All chemicals used were of reagent or higher grade. β-Carotene was from Sigma Chemical Co. (St. Louis, MO USA). Lycopene, AAPH, AMVN, iron nitrate anhydrate, nitrilotriacetic acid (NTA), and tetrahydrofuran (THF) were from Wako Co. (Osaka, Japan). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin, penicillin, streptomycin, sodium pyruvate, and non-essential amino acids (NEAA) were from GIBCO/BRL (Rockville, MD USA).

Cell culture

Hs68 cells were grown in DMEM containing 10% (v/v) FBS, 0.37% (w/v) $NaHCO_3$, penicillin (100 U/mL), streptomycin (100 μg/mL), 0.1 mM NEAA, 1 mM sodium pyruvate, and 5% CO_2 in an incubator at 37°C. The cells were harvested at approximately 90% confluence (10^6 cells/dish). The survival rate of cells was always higher than 95% by Trypan-blue assay.[27]

Carotenoid incorporation into cells

Both the concentrations (10 and 20 μM) and the method of incorporation of β-carotene and lycopene into Hs68 cells were adopted from those of Palozza et al.[10] A THF–carotenoid solution (5 mM) was prepared freshly before each experiment. Aliquots of THF (approximately 0.4%) or THF–carotenoid were added to Hs68 cells (approximately 10^6 cells/dish) and incubated in 10 mL of 1 × Hank's balanced salt solution (HBSS) buffer, pH 7.4, at 37°C in the dark for 1 hr. The cells were then washed in phosphate buffered saline (PBS), pH 7.4, for 3 times and the concentrations of β-carotene and lycopene were determined at 450 nm and 470 nm, respectively, following extraction in ethanol and hexane (1:2; v/v), as described previously.[28]

Oxidative challenge

To induce oxidative damage, carotenoid-enriched or control Hs68 cells (approximately 10^6 cells/dish) were washed three times in PBS and incubated with an oxidant in 10 mL of 1 × HBSS buffer at 37°C in the dark. AMVN was dissolved in methanol, and the latter (at a final concentration of 5%) was always included in the control experiments. AAPH and Fe/NTA were dissolved in HBSS; and Fe/NTA was prepared by mixing 1 mM $Fe(NO_3)_3$ with 4 mM NTA just before the experiment.[29] The reaction was terminated by adding butylated hydroxytoluene (BHT) to a final concentration of 0.5 mM.

Measurement of lipid peroxidation and lactate dehydrogenase activity

Lipid peroxidation was measured as thiobarbituric acid-reactive substances (TBARS) released into the HBSS me-

dium from Hs68 cells following centrifugation at 1,000 *g* for 10 min. TBARS were measured by mixing equal volumes of the supernatant with 0.7% TBA reagent and 2.5% trichloroacetic acid (TCA).[30] Additional BHT (0.5 mM) was included to prevent sporadic lipid peroxidation during heating at 100°C for 10 min. TBARS were extracted with an equal volume (3 mL) of butanol and centrifuged briefly, and the fluorescence of the butanol layer was measured at 515 nm excitation and 555 nm emission.[31] The unit of TBARS was expressed as nmol malondialdehyde (MDA) equivalent/10^6 cells using 1,1,3,3-tetramethoxypropane as MDA standard. The percentage of release of lactate dehydrogenase (LDH) was used as an index of cytotoxicity, and the activity was measured spectrophotometrically using pyruvate as substrate.[32] Total LDH was measured in cell lysates obtained by treatment with 0.5% Trition X-100, and the percentage of release was determined by dividing the LDH activity in the medium by total LDH activity.

Measurement of 8-hydroxy-2′-deoxyguanosine (8-OH-dG)

DNA was isolated from Hs68 cells devoid of medium by centrifugation (10,000 *g*, 10 min) using phenol/chloroform/isoamyl alcohol.[33] An equal amount of DNA (200 μg) was then digested with nuclease P_1 and alkaline phosphatase, and the 8-OH-dG levels were analyzed by HPLC with an electrochemical detector (Bioanalytical Systems, model LC-4C, West Lafayette, IN USA)[34] as described previously.[35] Oxidative damage was expressed as the molar ratio of 8-OH-dG to 10^5 molecules of deoxyguanosine (dG), which was calculated from the absorbance at 260 nm.

Comet assay

Comet assay was adapted from the method of Singh et al.[36] After oxidant treatment, cells were suspended in low-melting-point agarose in PBS at 37°C and pipetted onto a frosted glass microscope slide precoated with a layer of 1% normal-melting-point agarose. After application of a third layer of 1% normal-melting-point agarose, the slides were immersed in cold-lysing solution (2.5 M NaCl, 100 mM EDTA, 10mM Tris, 1% sodium lauryl sarcosinate, 1% Triton X-100) for 1 hr at 4°C. The slides were then placed in an electrophoresis tank, allowing the DNA to unwind for 15 min in the alkaline solution (300 mM NaOH and 1 mM EDTA). Electrophoresis was then performed at 300 mA for 20 min in the same alkaline solution at room temperature. The slides were then neutralized with 0.4 M Tris-HCl buffer (pH 7.4) and stained with ethidium bromide. The image analysis was performed using the method of Collins et al.[37] One hundred comets on each slide were scored visually as one of five classes according to tail intensity and given a value of 0, 1, 2, 3, or 4. The total score for 100 comets ranged from 0 to 400.

Data analysis

Values are expressed as means ± SD and analyzed using one-way analysis of variance followed by Duncan's multiple range test or using Student's *t*-test when two groups were compared.

Table 1 Concentration effects of lycopene and β-carotene on levels of TBARS in Hs68 cells incubated with an oxidant*

Treatment	TBARS (nmol MDA equivalent/10^6 cells)†		
	Fe/NTA	AAPH	AMVN
Control	14.0 ± 0.9[a]	4.6 ± 1.1	4.2 ± 0.8[a]
+10 μM lycopene	12.7 ± 0.8[a]	4.5 ± 0.8	4.4 ± 0.9[a]
+20 μM lycopene	9.7 ± 0.7[b]	4.3 ± 0.9	5.9 ± 1.2[b]
+10 μM β-carotene	12.5 ± 1.1[a]	4.0 ± 1.2	3.9 ± 0.7[a]
+20 μM β-carotene	12.3 ± 0.8[a]	3.6 ± 1.1	5.7 ± 1.5[b]

*Cells were incubated with lycopene or β-carotene in the dark at 37°C for 1 hr and then washed before incubation with 1 mM Fe/NTA for 2 hr or with 50 mM AAPH and 50 mM AMVN for 4 hr.
†Values (means ± SD of 3 experiments) in a column sharing a common letter are not significantly different ($P > 0.05$).
TBARS–thiobarbituric acid-reactive substances. Hs 68 cells–human foreskin fibroblasts. MDA–malondialdehyde. Fe/NTA–ferric nitrilotriacetate. AAPH–2, 2'=azobis(2-amidinopropane)dihydrochloride. AMVN–2,2'=azobis(2,4-dimethylvaleronitrile).

Results

Lipid peroxidation

Table 1 shows the concentration effects of lycopene and β-carotene on oxidant-induced lipid peroxidation in Hs68 cells. In cells incubated with 1 mM Fe/NTA for 2 hr, enrichment with 10 and 20 μM β-carotene only slightly ($P > 0.05$) decreased TBARS (from 14 to 12.5 and 12.3 nmol MDA/10^6 cells, respectively) and did not exhibit concentration effect. However, 20 μM lycopene significantly decreased TBARS (from 14 to 9.7 nmol MDA/10^6 cells) induced by 1 mM Fe/NTA, and the effect of lycopene tended to be dose-dependent. Neither β-carotene nor lycopene significantly affected lipid peroxidation during 4 hr of incubation of cells with 50 mM AAPH. In contrast, both lycopene and β-carotene increased TBARS released from Hs68 cells incubated with 50 mM AMVN for 4 hr, and TBARS released from the lycopene and β-carotene-enriched cells were 40% ($P < 0.05$) and 36% ($P < 0.05$) higher than the AMVN control. The results show that lycopene was stronger than β-carotene in both the antioxidant and pro-oxidant effects.

To further characterize the antioxidant and pro-oxidant effects of lycopene, we then used 20 μM lycopene to study the time course of lipid peorxidation induced by Fe/NTA and AMVN. *Figure 1A* shows a consistent inhibition of 1 mM Fe/NTA-induced TBARS by lycopene during the 2-hr incubation. By contrast, enrichment of cells with lycopene enhanced release of TBARS from Hs68 cells during the entire 4-hr incubation with 50 mM AMVN (*Figure 1B*).

DNA damage

Comet images of Hs68 cells with or without treatments were classified visually as one of five classes of damage, that is, from 0 (no visible tailing) to 4 (head of comet very small, most of DNA in tail). Although visual scoring is subjective, it shows a clear relationship to the percentage of DNA appearing in the tail, as measured by computer image analysis.[36] Because DNA was damaged too extensively by 1 mM Fe/NTA, 50 mM AAPH, and 50 mM AMVN, the

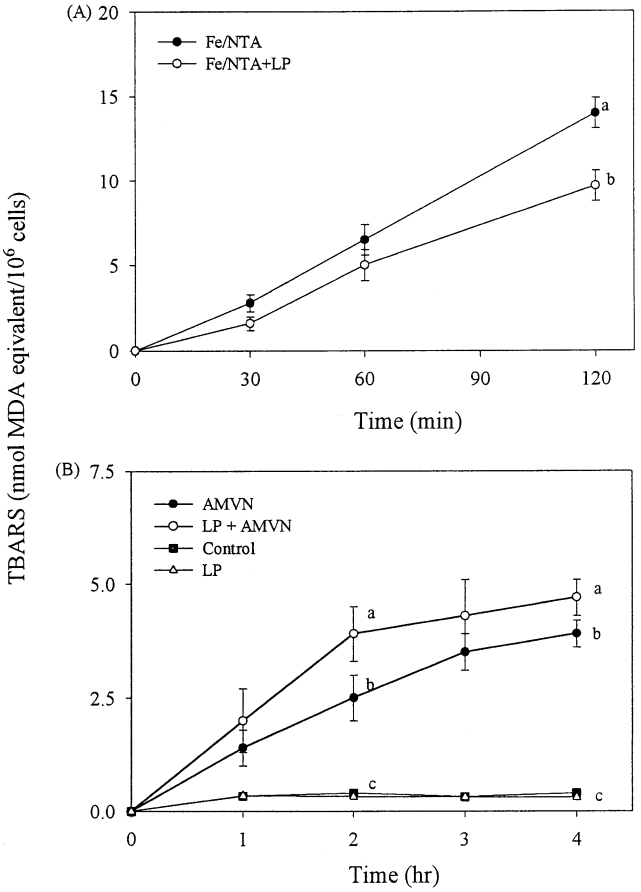

Figure 1 Time course of the effects of 20 μM lycopene (LP) on levels of thiobarbituric acid-reactive substances (TBARS) in human foreskin fibroblasts (Hs68 cells) incubated with 1 mM ferric nitrilotriacetate (Fe/NTA; A) or 50 mM 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN; B) at 37°C in the dark. Values (means ± SD of 3 experiments) at the same time points sharing a common letter are not significantly different ($P > 0.05$).

cells were incubated with lower concentrations of the oxidants. *Table 2* shows that the comet formation of Hs68 cells treated with oxidants for 1 hr was between Class 0 and 2 for 1mM AAPH, between Class 2 and 4 for 1 mM AMVN, and between Class 3 and 4 for 0.1 mM Fe/NTA. Both β-carotene and lycopene significantly increased comet formation in the absence of an added oxidant over the solvent control and blank. However, the extent of such damage was minimal, and there was no dose dependency for either lycopene or β-carotene; the scores were 85 and 87 for 20 and 40 μM lycopene, respectively, and 89 and 90 for 20 and 40 μM β-carotene, respectively (data not shown). Neither lycopene nor β-carotene significantly affected comet formation induced by any of the three oxidants used. Lycopene or β-carotene enrichment also did not significantly affect DNA damage measured as 8-OH-dG in cells incubated for 2 hr with 1 mM Fe/NTA, 50 mM AAPH, or 50 mM AMVN (*Table 3*).

Carotenoid uptake and consumption

Approximately 1–2% of the exogenous lycopene and β-carotene was incorporated into Hs68 cells after 1-hr incubation at 37°C in the dark. Hs68 cells enriched with 20 μM lycopene and β-carotene incorporated approximately 3.8 nmol lycopene/10^6 cells (or 6.3 nmol lycopene/mg protein) and 3.5 nmol β-carotene/10^6 cells (or 5.8 nmol β-carotene/mg protein), respectively. Oxidant incubation led to consumption of lycopene and β-carotene incorporated into Hs68 cells (*Figure 2*). The extent of loss of both lycopene and β-carotene by the oxidants was in the order of 50 mM AMVN, 1 mM Fe/NTA, and 50 mM AAPH. After incuba-

Table 2 Effects of lycopene and β-carotene on DNA damage measured as comet formation in Hs68 cells induced by oxidants*

Treatment	Arbitrary unit of comet assay†		
	Control	Lycopene (20 μM)	β-Carotene (20 μM)
Blank‡	40 ± 15	—	—
Solvent control§	46 ± 24[a]	85 ± 38[b]	89 ± 33[b]
Fe/NTA	308 ± 46[a]	264 ± 34[a]	284 ± 46[a]
AAPH	139 ± 26[a]	129 ± 26[a]	133 ± 27[a]
AMVN	287 ± 119[a]	272 ± 104[a]	277 ± 127[a]

*Cells were incubated with lycopene or β-carotene in the dark at 37°C for 1 hr and then washed before incubation with 0.1 mM Fe/NTA, 1 mM AAPH or 1 mM AMVN for 1 hr.
†Arbitrary unit is the total score of 100 comets ranging from 0 to 400 (each comet was scored visually as one of five classes according to tail intensity and given a value of 0, 1, 2, 3, or 4). Values (means ± SD of 3 experiments) in a row sharing a common letter are not significantly different ($P > 0.05$). Neither lycopene nor β-carotene enrichment significantly affected comet formation induced by any of the oxidants used.
‡Blank refers to the no-solvent control, that is, cells that have neither been incubated with a carotenoid or its solvent (THF) nor been incubated with an oxidant or its solvent (HBSS buffer or methanol).
§Solvent control refers to the cells incubated with THF (solvent for lycopene and β-carotene) for 1 hr at a final concentration 0.4%. The cells were then incubated with methanol (solvent for AMVN) for 1 hr at a final concentration of 5%. The solvent control and blank are not significantly different (Student's *t*-test, $P > 0.05$).
Hs68 cells–human foreskin fibroblasts. Fe/NTA–ferric nitrilotriacetate. AAPH–2,2'=azobis(2-amidinopropane)dihydrochloride. AMVN–2,2'=azobis(2,4-dimethylvaleronitrile). THF–tetrahydrofuran. HBSS–Hank's balanced salt solution.

Table 3 Effects of lycopene and β-carotene on DNA damage measured as 8-OH-dG content in Hs68 cells induced by oxidants*

Treatment	8-OH-dG/10^5 dG†		
	Control	Lycopene (20 μM)	β-Carotene (20 μM)
Control	n.d.	—	n.d.
Fe/NTA	5.8 ± 0.9	4.5 ± 0.5	5.8 ± 1.1
AAPH	6.1 ± 3.2	6.3 ± 0.9	5.2 ± 2.3
AMVN	9.1 ± 4.1	7.9 ± 2.0	12.5 ± 5.1

*Cells were incubated with lycopene or β-carotene in the dark at 37°C for 1 hr and then washed before incubation with 1 mM Fe/NTA for 2 hr or with 50 mM AAPH and 50 mM AMVN for 4 hr.
†Values are means ± SD of 3 experiments. Neither lycopene nor β-carotene resulted in significant differences in 8-OH-dG levels induced by any of the oxidants used ($P > 0.05$).
8-OH=dG–8=hydroxy=2'; = deoxyguanosine. Hs68 cells–human foreskin fibroblasts. Fe/NTA–ferric nitrilotriacetate. AAPH–2, 2'=azobis (2-amidinopropane)dihydrochloride. AMVN–2,2'=azobis(2,4-dimethylvaleronitrile). n.d.–not detectable (<1.5 8-OH-dG/10^5 dG).

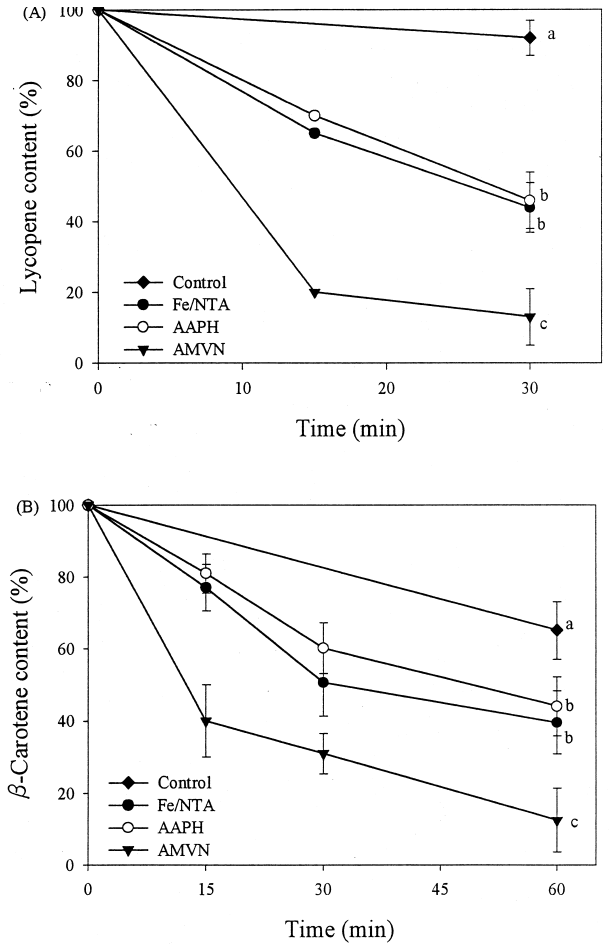

Figure 2 Consumption of incorporated lycopene (A) and β-carotene (B) in human foreskin fibroblasts (Hs68 cells) incubated in the dark with 1 mM ferric nitrilotriacetate (Fe/NTA), 50 mM 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH), or 50 mM 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN). Values (means of 2 experiments or means ± SD of 3 experiments) sharing a common letter are not significantly different ($P > 0.05$). The controls refer to the methanol control, that is, carotenoid-enriched cells incubated with methanol (the solvent for AMVN) at a final concentration of 5%. The solvent control and no-solvent control (i.e., Hank's balanced salt solution buffer to replace methanol) are not significantly different (Student's *t*-test, $P > 0.05$).

tion for 60 min, approximately 40% β-carotene remained in cells treated with 50 mM AAPH or 1 mM Fe/NTA, whereas only 10% β-carotene remained in cells incubated with 50 mM AMVN (*Figure 2A*). The loss of lycopene was faster than that of β-carotene in all three oxidants used. In cells treated with 50 mM AAPH, 1 mM Fe/NTA, and 50 mM AMVN for 30 min, 60%, 51%, and 31% β-carotene, respectively, remained (*Figure 2B*) but only 46%, 44%, and 13% lycopene, respectively, remained (*Figure 2A*). When the oxidants were replaced by methanol (i.e., solvent controls), approximately 92% lycopene remained after 30 min incubation (approximately 90% remained after 1-hr incubation), whereas only 65–70% β-carotene remained after 1-hr incubation. These figures were not significantly different between the solvent control and the no-solvent control (i.e., methanol replaced by 1 × HBSS buffer; data not shown), indicating that 5% methanol does not remove the carotenoids from the cells by a solvent effect.

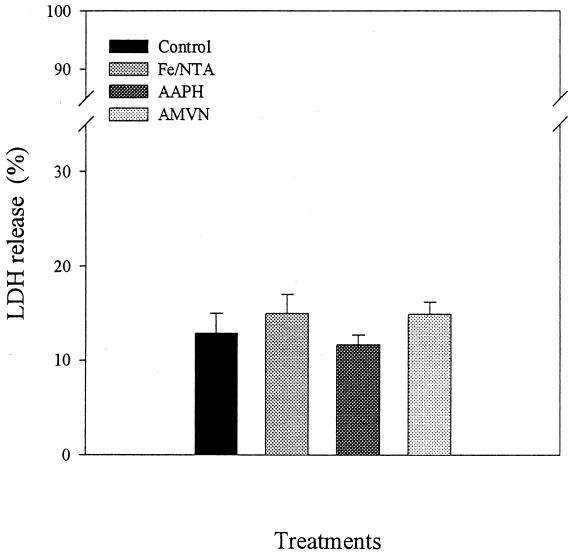

Figure 3 Release of lactate dehydrogenase (LDH) of human foreskin fibroblasts (Hs68 cells) incubated with oxidants. The cells were not pre-incubated with lycopene or β-carotene but were incubated for 4 hr with 50 mM 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) or 50 mM 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) or for 2 hr with 1 mM ferric nitrilotriacetate (Fe/NTA). Values (means ± SD of three experiments) are not significantly different ($P > 0.05$) from the solvent (methanol) control or from the no-solvent control (Hank's balanced salt solution buffer, data not shown).

Cell morphology and cytotoxicity

The cell morphology was markedly changed when the cells were incubated with 1 mM Fe/NTA or 50 mM AMVN at 37°C for 1 hr, but were not affected by 50 mM AAPH for up to 4 hr (data not shown). However, incubation of cells for 4 hr with 50 mM AAPH or 50 mM AMVN, or for 2 hr with 1 mM Fe/NTA did not increase cell lysis because the percentages of LDH released into the medium were not significantly different from that of the solvent control (*Figue 3*). Enrichment of the cells with either β-carotene or lycopene did not affect cell morphology (data not shown).

Discussion

The present study employed Hs68 cells under oxidative stress to investigate the antioxidant and/or pro-oxidant effects of lycopene because few studies have used cultured cells for these studies and, in particular, for comparison with β-carotene. A recent study in normal and tumor thymocytes showed that β-carotene is an antioxidant at low oxygen pressure but a pro-oxidant at high oxygen concentrations.[10] Our results from Hs68 cells at normal oxygen tension (under 95% air) demonstrated that lycopene and β-carotene can be either antioxidant or pro-oxidant depending on the type of oxidants used and that these effects of lycopene are somewhat stronger than those of β-carotene. The finding that lycopene also behaved as an pro-oxidant in Hs68 cells treated with AMVN is somewhat surprising because this carotenoid has been shown to be a better antioxidant than β-carotene.[18–21] Thus, the two carotenoids are similar in their actions under oxidative stress. This similarity may be

related to the fact that they contain the same polyene chain consisting of 11 conjugated double bonds—an important radical-scavenging structure of both compounds.[6,38]

It is unclear how β-carotene and lycopene may enhance lipid peroxidation in Hs68 cells. However, two theories have been proposed to explain the possible pro-oxidant effects of β-carotene in smokers. One theory holds that β-carotene forms radical cation by regenerating vitamin E radical and that vitamin C is important in reducing the radical cation of β-carotene.[39,40] It is well established that cigarette smokers have lower serum levels of vitamin C than do nonsmokers and, hence, smokers may not have enough vitamin C to recycle the radical cation of β-carotene, which could damage important cellular macromolecules including DNA.[39,40] However, because β-carotene is highly lipophilic and is located at the interior membrane, the water-soluble antioxidants such as vitamin C cannot efficiently reduce the carotene radicals. Thus, a second theory appears more likely, in that β-carotene may react with lipid peroxyl radical and form radical cation, which can then be reduced by vitamin E.[41,42] Vitamin C, in turn, can reduce vitamin E radical to regenerate vitamin E and thus prevent the damaging effects of β-carotene cation.[41] In this context, it has been suggested that nutritional intervention using a combination of antioxidants including β-carotene, vitamin E, and vitamin C could be a rational approach to reduce cancer risk.[14] The second theory may hold true for lycopene because the compound is extremely lipophilic and its reaction with lipid radicals may be confined to hydrophorbic inner core.[1] In accord with this theory, we showed here that the consumption of lycopene and β-carotene was much more extensive by the lipid-soluble AMVN than by the water-soluble AAPH and Fe/NTA. The carotenoid consumption by the oxidants was neither due to a solvent effect (e.g., 5% methanol did not cause carotenoid loss) nor to cell lysis because none of the oxidants significantly increased the percentage of LDH release (*Figure 3*), although AMVN and Fe/NTA induced evident changes in cell morphology.

Little information exists regarding the effect of lycopene on oxidative DNA damage in vitro. A recent study using the comet assay to study DNA damage in HT29 cells induced by xanthine/xanthine oxidase showed that both lycopene and β-carotene protect against DNA damage at relatively low levels (1–3 μM) but rapidly lose this capacity and, in fact, may have enhanced such damage at higher concentrations (4–10 μM).[43] These results are somewhat different from ours because we found no significant effects of lycopene and β-carotene on oxidant-induced DNA damage measured as comet formation. This discrepancy may have been due to several factors such as different cells, different oxidative systems, and slightly different concentrations of lycopene and β-carotene. However, our results from 8-OH-dG assay also showed that lycopene and β-carotene did not significantly effect DNA damage induced by the three oxidants used. 8-OH-dG is a sensitive marker of DNA damage[6,33] and, unlike the comet assay, is a quantitative measure of DNA damage. As mentioned earlier (in the Introduction section), results from ex vivo studies on the effects of β-carotene on DNA damage are also mixed.[12,13] Thus, whether lycopene and β-carotene affect oxidative DNA damage remains to be resolved. We found in this study that there was a slight but not dose-dependent increase in comet formation induced by both lycopene and β-carotene without oxidant treatment. This slight increase may be attributed to the presence of some pre-formed auto-oxidative products or the products formed during incubation (unpublished observation).

A question that may be asked is whether the concentrations used in the present in vitro study have any bearing in vivo. The mean human serum concentrations, which vary widely in different populations, range from 50 to 900 nM.[1] Thus, the concentrations (10–20 μM) used in the present study and by others[10] seem rather high. However, it is possible that the serum lycopene concentration may be greatly increased by high intake of tomatoes and their products, which are the main dietary source of lycopene,[1] and by nutritional supplements that contain lycopene. In fact, our results may have some relevance in vivo when comparing the levels of lycopene and β-carotene incorporated into Hs68 cells with the endogenous levels in human LDL. In the present study, approximately 1–2% of the 20 μM carotenoids, that is, approximately 3.8 nmol lycopene/10^6 cells (or 6.3 nmol/mg protein) and 3.5 nmol β-caroten/10^6 cells (or 5.8 nmol/mg protein) was incorporated into Hs68 cells in 1 hr. These levels are similar to or slightly higher than the endogenous levels of lycopene and β-carotene in isolated human LDL (0.37–2.0 nmol lycopene and 0.22–0.29 nmol β-carotene/mg protein in three subjects). [22] Thus, it is possible that these cellular levels of lycopene and β-carotene are sufficient to exert antioxidant and/or pro-oxidant effects in vivo.

In summary, the present study in Hs68 cells demonstrates that both lycopene and β-carotene can be antioxidant or pro-oxidant depending on the types of oxidants used. Neither lycopene nor β-carotene affected DNA damage induced by Fe/NTA, AAPH, or AMVN. The similar effects of lycopene and β-carotene may be attributed to the similarity in their chemical structures. The results suggest that lycopene may not be an ideal replacement for β-carotene and that it may be premature to conduct clinical trials on lycopene judging from the results of recent clinical studies on β-carotene.

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