

Lycopene in Association with α -Tocopherol Inhibits at Physiological Concentrations Proliferation of Prostate Carcinoma Cells

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The effect of lycopene alone or in association with other antioxidants was studied on the growth of two different human prostate carcinoma cell lines (the androgen insensitive DU-145 and PC-3). It was found that lycopene alone was not a potent inhibitor of prostate carcinoma cell proliferation. However, the simultaneous addition of lycopene together with α -tocopherol, at physiological concentrations (less than 1 μ M and 50 μ M, respectively), resulted in a strong inhibitory effect of prostate carcinoma cell proliferation, which reached values close to 90 %. The effect of lycopene with α -tocopherol was synergistic and was not shared by β -tocopherol, ascorbic acid and probucol. © 1998 Academic Press

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A recent study has shown an inverse relationship between dietary intake of lycopene-rich food and the risk of prostate cancer. Other dietary carotenoids such as β -carotene, α -carotene, lutein and β -cryptoxanthin did not show the same correlation (1). Also high α -tocopherol intake has been associated, in epidemiological studies (2, 3) with a strong decrease of prostate cancer risk (30-35%). In this study the question has been asked whether lycopene alone or in association with α -tocopherol or with other antioxidants affects the growth of two different human prostate carcinoma cell lines.

MATERIALS AND METHODS

Materials

Synthetic lycopene (95%, all *E*) was donated by F. Hoffmann-La Roche Ltd. (Basel, Switzerland), DL- α -tocopherol and DL- β -tocopherol were from Merck KGaA (Darmstadt, Germany), L-ascorbic acid and probucol were from Sigma Chemie (Switzerland). Tetrahydrofuran (THF) containing 0.0025% di-*t*-butyl-*p*-cresol as stabilizer was purchased from Fluka Chemie (Buchs, Switzerland)

and used as a solvent for the carotenoid. At the tested concentrations (maximal 0,5 %) THF was without effect on cell viability.

Carotenoid Solutions

Crystalline lycopene was maintained at -20°C and fresh 1 mM solutions in THF were prepared under dark room conditions just before the starting of experiments and added at the desired concentrations to the culture medium under vigorous stirring. Final concentration of lycopene in the medium was determined spectrophotometrically after its extraction with 2-propanol and *n*-hexane/dichloromethane (4). α -tocopherol, β -tocopherol and probucol were added as ethanol solutions (final ethanol concentration: 0.1%), ascorbic acid was added as an aqueous solution.

Cells

Culture media and antibiotics were from Gibco (Grand Island, NY, USA). Fetal calf serum was from PAA Labor (Linz, Austria). Human prostate carcinoma, androgen insensitive, cell lines DU-145 and PC-3, colon carcinoma (Caco-2), hepatoma (HepG2) and rat aortic smooth muscle cells (A7r5) were from American Type Culture Collection (Rockville, Maryland, USA). DU-145 cells were grown in minimum essential medium (MEM), PC-3 cells in F-12K medium, all of them containing antibiotics and 10% fetal calf serum (FCS). Caco-2 cells were grown in MEM containing 20% FCS, HepG2 cells were grown in MEM containing 10% FCS, A7r5 cells were grown in DMEM containing 10% FCS.

Cell Proliferation Assay

The proliferation was determined using two methods, cell count and thymidine incorporation:

Cell count. Cells were seeded into 6-multiwell plates (20,000 cells/well). When cells reached approximately 50% confluence the indicated concentrations of the compounds were added. THF was added to control cells to account for the effect of the solvent. Cells were washed, trypsinized, resuspended and counted in duplicated at the indicated times by trypan blue dye exclusion with an hemocytometer. Usually cells received only a single dose of the compounds except where indicated. Cells between passage 5 and 20 were used for all the experiments.

Thymidine incorporation. Colon carcinoma cells (Caco-2) and hepatoma cells (HepG2) seeded into 6-multiwell plates (30,000 cells/well) after treatment were pulsed with [^3H]thymidine (0,5 μ Ci per well) for two hours. After labeling, cells were washed with PBS +

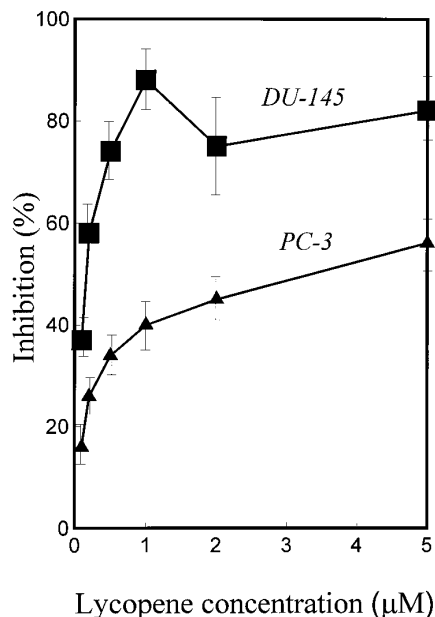


FIG. 1. Proliferation of lycopene plus α -tocopherol-treated DU-145 (A) and PC-3 (B) cells. Cells were stimulated to grow in media containing the indicated concentrations of lycopene plus α -tocopherol and compared with an equal volume of solvent-supplemented cells. Proliferation was measured at the indicated times and values were expressed as percentages of control, untreated cells. Results are means \pm SD of three independent experiments.

0.1% BSA + 1% glucose, fixed for 20 minutes with ice-cold 5% trichloroacetic acid and solubilized in 0.1 M NaOH/2% Na₂CO₃/1% SDS. The radioactivity incorporated into the acid-insoluble material was determined in a liquid scintillation analyzer.

RESULTS AND DISCUSSION

In a study preparatory to the present one, lycopene, at concentrations of 50 μ M or higher, has been used to inhibit the proliferation of different prostate carcinoma cell lines. However, since the lycopene concentration present physiologically in plasma is about 0.7 μ M (5) we do not attribute to the inhibition obtained at 50 μ M lycopene a physiological significance and those data are not shown here. However, lycopene at the concentrations of 1 μ M, in association with 50 μ M α -tocopherol, inhibited the proliferation of the two different prostate carcinoma cell lines used in this study (Figure 1). DU-145 proliferation in presence of 1 μ M lycopene and 50 μ M α -tocopherol was 88% inhibited, PC-3 proliferation was 40% inhibited. The inhibition was dose-dependent and increased at higher lycopene concentrations.

The effect of lycopene and α -tocopherol association in inhibiting cell proliferation was not an additive effect. As shown in Figure 2A a synergistic phenomenon was observed when both compounds were added together. At a constant α -tocopherol concentration of 50 μ M and

lycopene concentrations ranging from 0.1 to 5 μ M, the degree of synergism in the inhibition of cell proliferation was increased starting from a lycopene concentration of 0.2 μ M and it reached its maximum value at 1 μ M (Figure 2B).

In order to establish whether the synergistic inhibition of α -tocopherol and lycopene was due to the antioxidant properties of the former, possibly preventing the destruction of the latter, the effect of other antioxidants was investigated on DU-145 cell proliferation. These compounds, β -tocopherol, ascorbic acid and probucol were tested alone at 50 μ M and in association with 1 μ M lycopene. The association of these compounds with lycopene (Figure 3A and B) showed no synergistic effect. Also the triple combination of lycopene, α -tocopherol and ascorbic acid did not potentiate the lycopene plus α -tocopherol inhibitory effect on prostate cells (data not shown).

The inhibition of proliferation by lycopene in association with α -tocopherol was more potent with the prostate cell line employed in this study than with a number of other lines (Table 1). The data show that DU-145 line is two to three times more inhibited by the lycopene- α -tocopherol combination than the other cells. Moreover, the difference between the value of the inhibition obtained at 1 μ M lycopene with that in the absence of lycopene (produced by the presence of α -tocopherol) gives the inhibition by lycopene alone. This inhibition was also the largest for DU-145 (48%) relative to the other lines HepG2, Caco-2 and A7r5 showing inhibition by lycopene of 19, 10 and 1%, respectively.

Lycopene, which is present in high amount in tomatoes and tomato-derived products, is the most efficient singlet quencher oxygen among carotenoids (5) and has potent antioxidant properties. Lycopene was found to be concentrated also in other body tissues, such as liver, adrenals and adipose tissue (6) as well as in the prostate (7). The presence of lycopene in the prostate (7) has suggested the hypothesis that lycopene may have direct effects within the prostate and contribute to the reduced prostate cancer risk observed in individuals who consume high amounts of tomato-based, lycopene rich foods (1, 7).

In vitro culture studies have shown a growth inhibitory effect of lycopene on mammary, lung and endometrial cancer cell proliferation (8). Additional roles of lycopene such as induction of the gap-junction communication between cells through the increased synthesis of connexin 43 were found (9). This effect has been associated with the ability of lycopene to restore the loss of gap junctions occurring in malignant processes.

In biochemical studies it has been also shown that cell proliferation is inhibited by α -tocopherol in certain sensitive cell lines (10-13).

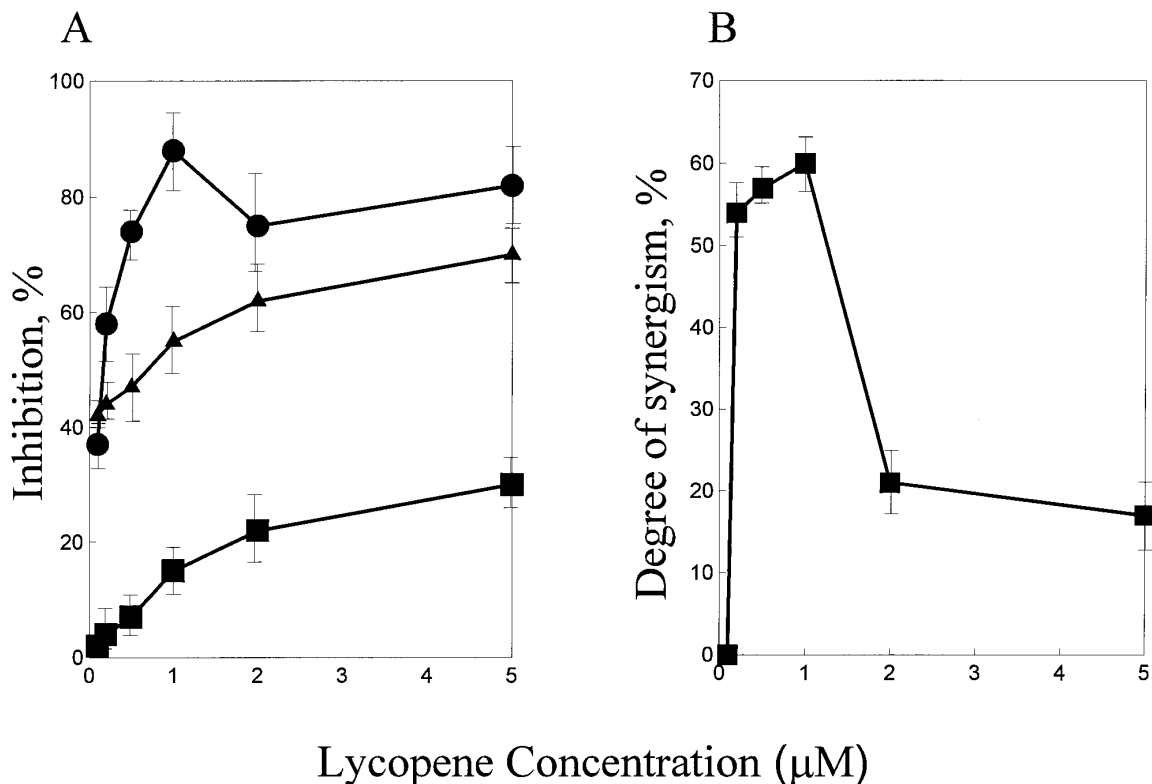


FIG. 2. (A) Effect of the association of α -tocopherol and lycopene on DU-145 cell proliferation. (B) Calculated degree of synergism between lycopene and α -tocopherol as a function of lycopene concentration. Cells (DU-145), in exponentially growing phase, were treated with lycopene alone (square symbols) or with the combination of α -tocopherol and lycopene at the indicated concentrations. The expected and the obtained inhibition values for the association between lycopene and α -tocopherol are represented as triangles and circles, respectively. Proliferation was measured as described in Materials and Methods. Data are expressed as percentage of inhibition obtained in treated relative to untreated cells taken as control. Values are means \pm SD of five independent experiments.

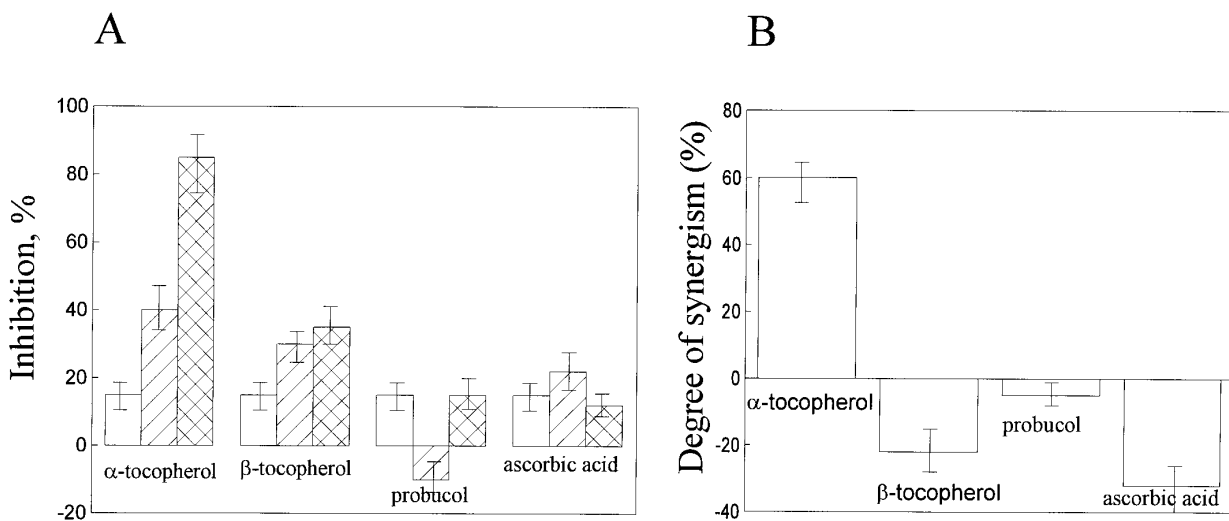


FIG. 3. (A) Effect of the concomitant addition of α -tocopherol, β -tocopherol, probucol, or ascorbic acid together with lycopene on DU-145 cell proliferation. (B) Calculated degree of synergism between lycopene and the indicated compounds. Cells (DU-145) were treated either with lycopene alone (empty bars) or with the indicated compounds (hatched bars) or with the association of them *plus* lycopene. Cell proliferation was determined as described in Materials and Methods. Results are means \pm SD from a representative of three separate experiments.

TABLE 1

Effect of the Association between Different Concentration of Lycopene and α -Tocopherol on the Proliferation of Different Types of Cell Lines

Cell line	Lycopene concentration, μ M				
	0	0.1	0.2	0.5	1
DU 145	40 \pm 6	37 \pm 4	58 \pm 6	74 \pm 9	88 \pm 8
HepG2	5 \pm 1	6 \pm 1	18 \pm 3	22 \pm 4	24 \pm 4
Caco-2	25 \pm 3	18 \pm 3	26 \pm 5	33 \pm 2	35 \pm 3
A7r5	40 \pm 5	14 \pm 2	28 \pm 3	37 \pm 4	41 \pm 2

Note. The values are expressed as % of inhibition. Prostate cancer cells (DU-145), hepatoma cells (HepG2), colon carcinoma cells (Caco-2) and rat aortic smooth muscle cells (A7r5) were treated with different concentrations of lycopene for 24 h. The inhibition in the absence of lycopene is produced by the presence of 50 μ M α -tocopherol. Proliferation was assessed as indicated in Materials and Methods. Data are expressed as percentage of inhibition compared to control. Data are means \pm SD of five experiments in duplicate.

Here we have shown that α -tocopherol is capable alone, at physiological concentrations, to produce growth inhibition of prostate tumor cells. Such an effect may be at the basis of the epidemiological studies that have indicated in vitamin E a nutrient capable of partially protecting against the risk of prostate cancer (14, 15). The strong potentiation by very low concentrations of lycopene of prostate tumor cell growth inhibition observed here may be at the basis of the other epidemiological studies linking high tomato consumption with low prostate cancer risk (1, 7). Among the cell lines studied here, prostate derived cell lines have shown to be more sensitive than all other tested lines to the combined effect of α -tocopherol and lycopene. The origin and the molecular basis of this specificity are at the basis of current investigations.

The roles of vitamin E and lycopene in the production of the synergistic inhibition studied here is not clear. It appears however that the inhibition is not related with an antioxidant mechanism, since several potent antioxidant were not capable of mimicking the α -tocopherol effect. It is possible that α -tocopherol and lycopene by affecting different signal transduction

pathways (9, 10) lead to synergistic inhibition of cell proliferation.

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