Nutrition and Disease

Combined Lycopene and Vitamin E Treatment Suppresses the Growth of PC-346C Human Prostate Cancer Cells in Nude Mice

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ABSTRACT   Epidemiologic studies have repeatedly associated a high intake of lycopene and vitamin E with reduced prostate cancer risk. The present study examined the ability of the 2 compounds to reduce tumor growth and prostate-specific antigen (PSA) plasma levels in the PC-346C orthotopic mouse model of human prostate cancer. Three days after intraprostatic tumor injection, NMRI nu/nu mice were administered a daily oral dose of synthetic lycopene [5 or 50 mg/kg body weight (BW)], vitamin E in the form of α-tocopheryl acetate (5 or 50 mg/kg BW), a mixture of lycopene and vitamin E (5 mg/kg BW each), or vehicle. Intraprostatic tumor volume and plasma PSA concentrations were measured at regular intervals. Mice were killed when the tumor load exceeded 1000 mm^3 or on d 95 when the study was terminated. Prostate and liver were analyzed by HPLC for lycopene isomers and α- and γ, δ-tocopherol concentrations. None of the single treatments significantly reduced tumor volume. In contrast, combined treatment with lycopene and vitamin E, at 5 mg/kg BW each, suppressed orthotopic growth of PC-346C prostate tumors by 73% at d 42 ($P < 0.05$) and increased median survival time by 40% from 47 to 66 d ($P = 0.02$). The PSA index (PSA:tumor volume ratio) did not differ between experimental groups, indicating that PSA levels were not selectively affected. Lycopene was detected only in mice supplemented with lycopene. As in humans, most tissue lycopene was in the cis-isomer conformation, whereas 77% trans-lycopene was used in the dosing material. Liver α-tocopherol concentrations were increased in mice supplemented with both 50 mg/kg (226%, $P < 0.05$) and 5 mg/kg vitamin E (41%, $P < 0.05$), whereas prostate α-tocopherol concentrations were increased only by the higher dose (83%, $P < 0.05$). Our data provide evidence that lycopene combined with vitamin E may inhibit the growth of prostate cancer and that PSA can serve as a biomarker of tumor response for this treatment regimen.   J. Nutr. 136: 1287–1293, 2006.

KEY WORDS: • prostate cancer • lycopene • vitamin E • chemoprevention • tumor xenograft model

Prostate cancer has emerged as a major public health issue in developed countries, where it is a leading cause of male malignancy (1). Given that the 3 established risk factors for prostate cancer, i.e., older age, a family history of the disease, and race, are nonmodifiable, the epidemiologic evidence that dietary and lifestyle factors are contributing factors to prostate cancer has prompted a search for safe foods and micronutrients that may lower prostate cancer risk (2–5). Among these, vitamin E and lycopene, a carotenoid found primarily in tomatoes, were identified as promising phytochemicals in the prevention and/or control of prostate cancer (4–11). Both micronutrients have a wide range of in vitro antitumor properties (12–15), but their actual benefits as agents for prostate cancer have not been firmly established in vivo.

Interest in a role of vitamin E in prostate cancer chemoprevention was sparked by the large randomized Finnish ATBC trial assessing α-tocopherol, a form of vitamin E, and β-carotene

for the prevention of lung cancer among smokers. Secondary end point data showed an unexpected strong reduction in prostate cancer incidence and mortality among participants receiveing α-tocopherol (6). However, subsequent prospective studies did not support an overall risk reduction of prostate cancer, but suggested a benefit limited to smokers (7,16–19). In animal models, vitamin E was efficacious against high fat–promoted prostate cancer growth (20), but lacked chemopreventive effects in rat prostate carcinogenesis models (21,22).

Mounting epidemiologic evidence over the past decade suggests that a high intake of tomato products or lycopene, the major carotenoid in tomato, might reduce the occurrence or progression of prostate cancer (7–11,23–25). Short intervention studies support a beneficial role of lycopene-rich tomato products in treating existing prostate cancer by showing reduced oxidative DNA damage (26) and PSA (prostate-specific antigen) blood levels (26–28) in supplemented prostate cancer patients. Although promising, these data do not prove a true antitumor effect of lycopene. First, given that some compounds directly affect PSA without affecting tumor growth (29,30), the reliability of PSA as a marker of tumor burden has yet to be

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confirmed for tomato products and lycopene. Second, it is unclear whether lycopene accounts for the observed benefits or whether it contributes to the effects of tomatoes by acting cooperatively with other components, such as phytofluene (31) or vitamin E (9,31–33). In support of the latter possibility, it was shown that simultaneous addition of lycopene and α-tocopherol at physiological concentrations led to a synergistic inhibition of prostate carcinoma cell proliferation (32).

The present study was undertaken to examine *1*) whether synthetic lycopene or vitamin E, individually or combined, can inhibit growth of prostate cancer, and *2*) whether PSA is a reliable marker with which to monitor these effects. The orthotopic PC-346C prostate xenograft model, developed at our laboratory (34,35), was chosen to investigate these issues. It consists of the human androgen responsive, PSA expressing, PC-346C cell line inoculated into the dorsolateral prostate of athymic nude mice. This model allows investigation of organ-specific chemopreventive effects through monitoring of in situ tumor growth by transrectal ultrasonography and simultaneous recording of plasma PSA levels (35–37). Because the uptake of lycopene and α-tocopherol in the prostate is likely instrumental in a tumor effect and little is known about the bioavailability of these nutrients in mice, we also measured lycopene isomer and α-tocopherol concentrations in liver and prostate.

MATERIALS AND METHODS

The PC-346C cell line.   The human prostate cancer cell line PC-346C was established from an athymic nude mouse–supported xenograft, PC-346, developed in our laboratory from a nonprogressive prostate tumor obtained by transurethral resection (34,37). Both parental xenograft and cell line are androgen responsive, harbor the wild-type androgen receptor, and release PSA. Passage 36 PC-346C cells, propagated under standard conditions (37) and grown to 70% confluence, were used for tumor inoculation.

Animals, diet, and housing.   Intact male NMRI nu/nu mice, 6 wk old ($n = 54$), specified pathogen free according to the Federation of European Laboratory Animal Science Association norm (38), were obtained from Harlan. Upon receipt, the mice were fed a 821077 CRM(P) low vitamin E rodent diet (Special Diets Services Witham). This diet is identical to the standard nonpurified 801722 CRM(P) diet generally used in our experiments (proximate composition: crude protein, 18.4%; crude oil, 3.4%; crude fiber, 4.2%; ash, 6.3%; nitrogen free extract 57.4%; total dietary fiber, 15.1%; moisture, 10%; digestible energy, 12.3 MJ/kg), except that the vitamin E concentration was reduced to 50 mg/kg feed (instead of 103 mg/kg feed). In this way, the mice received sufficient amounts of vitamin E, and the possibility of a confounding effect of vitamin E in the basal diet on the effects of the supplementation was minimized. Irradiated chow and acidified drinking water were consumed ad libitum. Mice were kept in $14 \times 13 \times 33.2$ cm^3 individually ventilated cages (Techniplast) with 3 mice/cage, on sawdust (Woody-Clean, type BK8/15; BMI) under a 12-h light:dark cycle, at $50 \pm 5\%$ relative humidity, in a temperature controlled ($\sim 22°C$) room. The experiment was approved by the Animal Experimental Committee (DEC) of Erasmus University and performed in agreement with The Netherlands Experiments on Animals Act (1977) and the European Convention for protection of Vertebrate Animals used for Experimental Purposes (Strasbourg, 18 March 1986).

Lycopene and vitamin E source.   The products used were those commonly used in human nutrition. Lycopene was provided as LycoVit® 10% (BASF Aktiengesellschaft), containing microencapsulated synthetic lycopene, with an analyzed content of 11.45% total lycopene (77% *all-trans*- and 23% total *cis*-lycopene) and <2% vitamin E . All-*rac*-α-tocopheryl acetate 50% powder (BASF Aktiengesellschaft) was used as the source of supplemental vitamin E. Both vitamin E and LycoVit 10% were dispersed in water in the appropriate concentrations. Stocks were freshly prepared each week and kept in the dark at 4°C. The stability of the solution was confirmed.

Experimental design.   Upon arrival, mice were randomly assigned to 1 of 6 groups ($n = 9$/group). After 2 wk of acclimation (d 0), mice were injected with 10^6 tumor cells into the dorsolateral prostate as described (39). Three days after tumor inoculation (d 3) mice were supplemented orally once each day according to the treatment they were assigned to: placebo (autoclaved water); lycopene [5 mg/kg BW (body weight)]; lycopene (50 mg/kg BW); vitamin E (5 mg/kg BW); vitamin E (50 mg/kg BW); lycopene + vitamin E (5 mg/kg BW each).

Intraprostatic tumor growth was monitored 1 time/wk by transrectal ultrasonography using an intravascular ultrasound system adapted for use in mice (35,36). Blood was obtained every 2 wk and at the time of killing through retroorbital puncture and collected in a heparin tube (Sarstedt). Plasma, obtained from blood after centrifugation for 5 min at $1500 \times g$, was used for PSA determination.

During the treatment period, all mice were weighed weekly and monitored daily for any overt sign of morbidity. Mice losing >15% of weight and/or mice having a tumor load exceeding 1000 mm^3 were killed by cervical dislocation after blood collection under anesthesia with diethylether (highest purity; Vel). The remaining mice were killed on d 95 when the study was terminated. The prostate (including tumor) and liver were immediately removed and weighed. The tissue was snap-frozen in liquid nitrogen and stored at $-80°C$ for HPLC analysis.

Measurement of plasma PSA levels.   Circulating plasma PSA levels were determined at the Department of Clinical Chemistry of Erasmus MC using an automated ELISA (Elecsys total PSA immunoassay; lower detection limit 2 ng/L; Roche Diagnostics).

Tissue extraction and HPLC analysis of lycopene and vitamin E. Tissue was homogenized with acetone:methanol (4:1) containing butylhydroxytoluol, cooled, excluding the influence of light or oxygen, and centrifuged (at 4°C for 10 min). After reextraction with acetone, the combined extracts were evaporated under inert gas and mixed with *n*-hexane and anhydrous sodium sulfate. An aliquot was evaporated, reconstituted with ethanol:1,4-dioxane:methanol (100:100:300, by vol), and subjected to HPLC analysis.

The HPLC system consisted of an Agilent 1100 apparatus equipped with an autosampler, a C30 carotenoid column (250×4.6 mm, 5 μm, YMC), a temperature controller, a diode array detector for lycopene (472 nm), a fluorescence detector for tocopherol (excitation: 295 nm, emission: 330 nm), and an Agilent ChemStation for data processing and evaluation. The HPLC mobile phase was methanol (solvent A) and methanol:methyl tert-butyl ether:tetrahydrofurane (140:800:60, by vol.; solvent B). A gradient was run at a flow rate of 1.0 mL/min (26°C) as follows: 100% solvent A for 12 min followed by a 35 min linear gradient to 100% solvent B; then a 2 min hold followed by a 3 min linear gradient back to 100% solvent A. Isomer distribution of lycopene was determined with an isocratic HPLC system using 2 C30 carotenoid columns arranged in series (15°C; 250×4.6 mm, a 5-μm and a 3-μm column, YMC) and UV detection (472 nm) The mobile phase was methanol:methyl *tert*-butyl ether:tetrahydrofurane 665:784:74, by vol.

Data analysis.   Mice without tumor take (tumor < 100 mm^3) ($n = 5$) were excluded from all analyses, except liver tissue analysis. Mice dying without prostate cancer as the evident cause ($n = 3$) were excluded from tumor volume and PSA analysis. In the survival analysis, non-tumor–related death was entered as a censored observation.

Data for tumor volume, PSA level, body weight change, lycopene, α- and γ/δ- tocopherol tissue concentration, and lycopene distribution were provided as means $\pm$ SEM. Mean tumor volumes and PSA were compared at d 42 only. For all variables, the Kolmogorov Smirnov goodness-of-fit test was used to determine whether the variable originated from a normal distribution. For normally distributed variables, differences between groups were compared using 1-way ANOVA, with the treatment group as a factor in the model. Homogeneity of variance was checked via Levene's test of homogeneity of variance. In case of homogeneity, the ANOVA *F*-test for statistical significance was used for testing equality of group means and Tukey's Honestly Significant Difference for making post hoc comparisons. In case of unequal variances, the Welch statistic was used for testing equality of group means and Dunnet's T3 for making post hoc comparisons. Differences were considered significant at $P < 0.05$. As was expected a priori, PSA levels did not follow a normal distribution. Therefore, ^{2}log(PSA) was used in the statistical analyses. Overall differences

between prostate and liver tissue levels (independent of treatment) were analyzed with the paired t test. The statistical analyses were performed using SPPS for Windows version 12.0.2.

Survival estimates were determined by the method of Kaplan and Meier. Survival data were compared by the nonparametric log-rank test. Survival time was defined as the day at death or euthanasia due to a tumor volume >1000 mm^3. Mice that survived until the end of the study with a tumor <100 mm^3 (tumor nontakers) were excluded from the survival analysis, whereas mice surviving until the end of the study with a tumor >100 mm^3 but <1000 mm^3 were entered as censored individuals. Deaths from causes unrelated to prostate cancer ($n = 3$) were also entered as censored data. Prism 4.0 software (GraphPad Software) was used to analyze the survival data and compose all figures.

RESULTS

General. Daily supplementation with lycopene and/or vitamin E through oral gavage was well tolerated and did not alter mean body weight change (**Table 1**). The only visible effect of treatment was a slight orange-red coloring of feces in mice administered lycopene. Weight loss occurred only in mice with a large tumor burden and in 3 mice that became ill and died for unknown reasons not obviously related to tumor burden or treatment. The mice were from 3 different groups (Table 1).

Of the 54 tumor-inoculated mice, 49 developed a prostate tumor (i.e., tumor volume >100 mm^3) within the 95-d study period (Table 1). This 91% tumor take rate is within the range normally observed in the PC-346C tumor xenograft model [(37) and W. van Weerden, unpublished observations].

Effect on orthotopic tumor growth and survival. Compared with the control, the combined treatment with a mixture of lycopene and vitamin E, at 5 mg/kg BW each, suppressed the growth of the prostate xenograft by 73% at d 42 ($P < 0.05$, **Fig. 1A**). As a consequence, mice supplemented with the lycopene-vitamin E mix survived longer than the controls ($P = 0.02$, log rank), with the median survival time increasing by 40% from 47 to 66 d (**Fig. 2**). In contrast, treatment with lycopene or vitamin E, at 5 or 50 mg/kg/BW, did not affect tumor growth or survival (Fig. 1A, Fig. 2). There was, however, a trend for slower tumor growth (53% inhibition at d 42) and increased median survival time (19% from 47 to 56 d) among mice supplemented with 5 mg/kg lycopene alone ($P = 0.1$).

Effect on plasma PSA concentration. In vehicle-treated mice, PSA plasma levels increased in proportion to tumor burden (cf Figs. 1A and 1B). This is in agreement with our previous demonstration of comparable sensitivity of transrectal ultrasonography and plasma PSA determination (35). As seen for the tumor volume, the plasma PSA levels tended to be lowest in mice administered the vitamin E-lycopene mix ($P = 0.06$). The PSA-index (plasma PSA level divided by tumor volume), a parameter used to demonstrate selective effects on PSA vs. tumor growth (40), did not differ among the groups (data not shown), indicating that plasma PSA-levels were proportional to tumor size regardless of dietary treatment.

Tissue concentration and distribution of lycopene isomers and α-tocopherol. Lycopene was not detected in prostate and liver of mice administered placebo or vitamin E alone. Lycopene supplementation resulted in a dose-dependent accumulation of lycopene in liver and prostate (**Table 2**). Overall, liver lycopene concentrations (0.055 ± 0.012 nmol/g) were greater than those in prostate (0.010 ± 0.002 nmol/g) ($P < 0.001$). Although the all-*trans* form of lycopene was most abundant in the preparation used for supplementation (77%), *cis* isomers (i.e., the sum of 5-*cis* and other *cis*-lycopene isomers) were the predominant isomers in liver (67–72%) and prostate (67–77%) (**Table 3**). The proportion of 5-*cis* lycopene was higher in prostate ($56.7 \pm 1.4\%$) than in liver ($45.0 \pm 1.2\%$) ($P < 0.001$). Notably, the proportion of *cis*-lycopene isomers in the prostate increased at the expense of their all-*trans* counterparts in mice gavaged with the high (50 mg/kg BW) lycopene dose ($P < 0.01$).

Due to the presence of vitamin E in the diet (analyzed concentration: 50 mg/kg), unsupplemented mice had substantial concentrations of α-tocopherol in prostate (13.53 ± 1.01 nmol/g) and liver (12.98 ± 0.95 nmol/g) (Table 2). Therefore, the prostate α-tocopherol concentration was enhanced beyond those of control mice by oral gavage only with the high 50 mg/kg BW vitamin E dose (83%, $P < 0.05$), whereas the liver α-tocopherol concentration was enhanced by both 5 and 50 mg/kg vitamin E (41 and 226% respectively, $P < 0.05$). The liver α-tocopherol concentration also was enhanced by the high 50 mg/kg BW lycopene dose, containing up to 8 mg/kg vitamin E (55%, $P < 0.05$).

Because α-tocopherol supplementation may decrease plasma γ- and δ- tocopherol levels, which may potentially offset health benefits induced by α-tocopherol supplementation (41,42), we

TABLE 1

Effect of orally supplemented lycopene and vitamin E, given alone or in combination, on body weight changes and tumor take of PC-346C inoculated mice

Daily treatment (mg/kg BW)	Body weight change at d 42[1]	Mice without tumor take[2]	Non-tumor related–deaths	Mice available for further analysis[3]
	%		n	
Placebo	1.4 ± 2.8	1	0	8
Lycopene (5 mg/kg)	1.0 ± 1.4	0	0	9
Lycopene (50 mg/kg)	-4.1 ± 1.5	0	1	8
Vitamin E (5 mg/kg)	0.0 ± 2.5	1	0	8
Vitamin E (50 mg/kg)	0.6 ± 4.4	2	1	6
Lycopene + Vitamin E (5 mg/kg each)	0.1 ± 2.1	1	1	7

[1] Values are means $\pm$ SEM ($n = 8$–9) for d 42 compared with d 3 (treatment initiation). At d 3, the mean body weight was 32.7 ± 2.5 g ($n = 54$).

[2] Number of mice not developing a tumor (>100 mm^3) during the study period.

[3] Number of mice excluded those not developing tumors ($n = 5$) and those with nontumor burden–related morbidity ($n = 3$). Each group initially consisted of 9 mice.
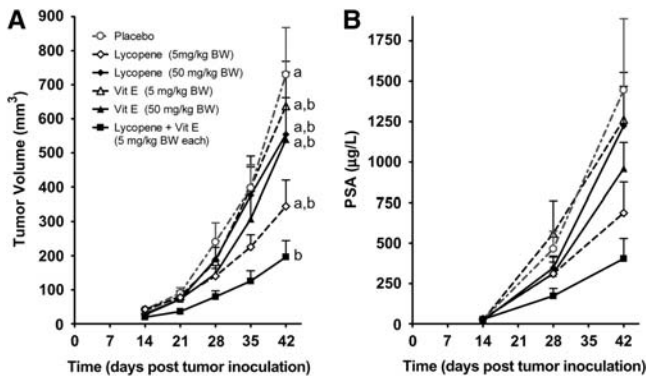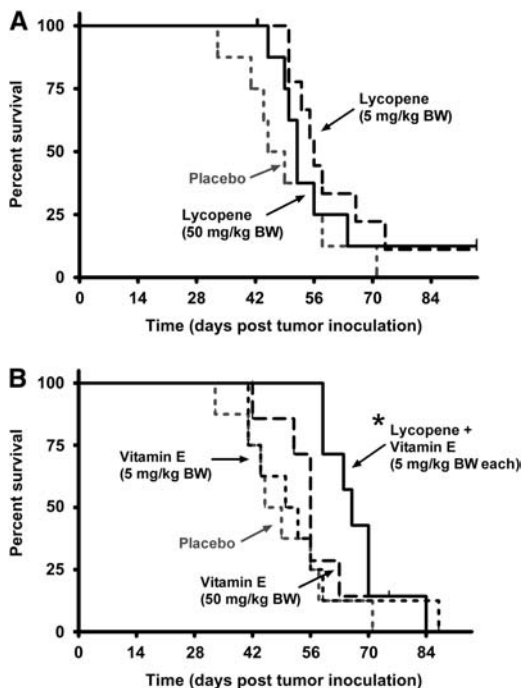
FIGURE 1 Effects of lycopene and vitamin E, alone or in combination, on tumor growth (*A*) and plasma PSA concentration (*B*) in NMRI nude mice inoculated intraprostatically with PC-346C human prostate cancer cells. Values are means ± SEM (*n* = 6–9 mice with tumors). Because death in the placebo group started to occur around d 42, means were calculated until then. Means at d 42 without a common letter differ (*P* < 0.05).

also measured γ,δ-tocopherol levels in liver tissues and in the few prostate samples available (Table 2). Neither α-tocopherol nor any of the other treatments affected tissue γ,δ-tocopherol concentrations (~1.5 nmol/g).

DISCUSSION

Epidemiologic and clinical evidence suggests that vitamin E and the major tomato-carotenoid lycopene may be of value for

FIGURE 2 Effects of lycopene (*A*), vitamin E (*B*) or combined lycopene-vitamin E treatment (*B*) on the survival of PC-346C bearing NMRI nude mice; *n* = 9 mice/group were monitored for survival. Mice that did not develop tumors were excluded from the survival analysis (*n* = 5). Survival time was defined as the day of death or euthanasia due to a tumor >1000 mm³. Mice dying of a cause unrelated to prostate cancer (*n* = 3) or mice surviving until the end of the study with a tumor >100 mm³ were entered as censored individuals (appearing as ticks superimposed on the staircase). *Different from the placebo group, *P* < 0.01.

prostate cancer prevention and control, but causality has not been established (5–11,16,23–25). To date, animal studies have been inconclusive in proving antiprostate cancer activity of lycopene or vitamin E as single agents (10,11,20–22,33,43–48).

In the present orthotopic xenograft mouse study, lycopene and vitamin E were ineffective individually, but markedly reduced tumor growth when given in combination, suggesting a cooperative interaction. This would be in line with the reported in vitro synergy between vitamin E and lycopene with regard to their antioxidant action (49) and growth inhibitory effects on prostate carcinoma cells (32) and with their in vivo additive effects on androgen target gene expression and oxidative stress reduction in rat prostate tumors (46). In accordance, an antioxidant cocktail containing vitamin E, lycopene, and selenium blocked prostate cancer development in *Lady* transgenic mice. However, the relevance of the individual components was not addressed in that study (48).

Until recently, it was generally thought that the beneficial effects of tomato-based foods and lycopene-rich tomato extracts were mediated by lycopene. Although in vitro data support lycopene's anticancer role, evidence for its in vivo efficacy as a single-nutrient intervention is less convincing. Three animal dietary studies addressing prevention showed no or no consistent chemopreventive effect (43–45). Interestingly, in a large study conducted in rats, there was a nonsignificant 9% decrease in survival after treatment with synthetic lycopene compared with a significant 27% decrease after whole tomato powder (45). However, the apparent inadequacy of lycopene in that study might also reflect a dose-dependent effect of lycopene because a 10-fold higher dose was used in the lycopene supplement than in the tomato powder (50,51). Indeed, in vitro, the protection of lycopene against oxidative damage follows a U-shaped curve (52). This might also explain why the 5 mg/kg BW, but not the 10-fold higher lycopene dose, tended to enhance survival (*P* = 0.1) in our prostate xenograft study. Another 2 xenograft studies examining lycopene as a treatment found an effect on necrosis without a concurrent effect on tumor size in rats (46) and a significant inhibition of androgen-independent prostate cancer growth in mice (47). Taken together, the preclinical data support a modest therapeutic and possibly preventive effect of lycopene on prostate cancer. However, the optimal dose, form, and combinations of lycopene with other phytochemicals or tomato components warrant further investigation.

Our study does not support a beneficial effect of vitamin E on prostate tumor growth per se. This is in sharp contrast to the 41% reduction in mortality from prostate cancer among subjects receiving α-tocopherol in the ATBC trial (6). One intriguing possibility is that vitamin E is beneficial only under particular conditions. In the prospective trials showing a positive effect of α-tocopherol on prostate cancer, an inverse association was consistently observed only among smokers (6,7,16–19). Similarly, vitamin E was shown to inhibit necrosis in the rat Dunning prostate cancer model (46), and a high-fat diet promoted prostate tumor growth in mice (20); however, chemopreventive effects were lacking in other animal models (21,22). Another explanation for the current lack of effect of vitamin E is that the basal vitamin E levels in the cereal-based diet (50 mg/kg feed) might have blunted a positive effect. Indeed, prostate α-tocopherol levels were raised only by daily oral supplementation with 50 mg/kg BW, but not with 5 mg/kg BW vitamin E. The inefficacy of α-tocopherol cannot be explained by a displacement of γ-tocopherol (41,42) because supplementation did not affect γ-tocopherol tissue concentrations (Table 2).

Due to the daily oral supplementation with lycopene and vitamin E, the uptake of lycopene and α-tocopherol, respec-

TABLE 2

Lycopene, α-tocopherol, and γ,δ-tocopherol concentrations in prostate and liver of mice supplemented with varying doses of lycopene and/or vitamin E[1]

Daily treatment (mg/kg BW)	Prostate (tumor)			Liver		
	Total lycopene	α-Tocopherol	γ,δ-Tocopherol	Total lycopene	α-Tocopherol	γ,δ-Tocopherol
	nmol/g					
Placebo	0 ± 0[a] (6)	13.53 ± 1.01[a] (6)	1.63 ± 0.34 (2)	0 ± 0[a] (8)	12.98 ± 0.95[a] (8)	1.58 ± 0.07 (8)
Lycopene (5 mg/kg)	0.014 ± 0.004[ab] (8)	14.09 ± 0.69[ab] (8)	1.21 ± 0.06 (4)	0.069 ± 0.012[b] (9)	12.62 ± 1.08[a] (9)	1.48 ± 0.11 (9)
Lycopene (50 mg/kg)	0.038 ± 0.006[b] (6)	19.12 ± 2.84[ab] (5)	0.79 (1)	0.321 ± 0.104[ab] (8)	20.13 ± 0.95[b] (8)	1.37 ± 0.07 (8)
Vitamin E (5 mg/kg)	0 ± 0[a] (8)	16.22 ± 1.00[ab] (8)	1.19 ± 0.10 (4)	0 ± 0[a] (8)	18.33 ± 0.76[b] (8)	1.44 ± 0.11 (8)
Vitamin E (50 mg/kg)	0 ± 0[a] (6)	24.80 ± 2.40[b] (6)	0.91 ± 0.12 (2)	0 ± 0[a] (6)	42.28 ± 2.77[c] (6)	1.42 ± 0.17 (6)
Lycopene + Vitamin E (5 mg/kg each)	0.012 ± 0.002[b] (7)	14.41 ± 0.72[ab] (7)	1.07 ± 0.11 (2)	0.058 ± 0.005[b] (7)	19.91 ± 0.85[b] (7)	1.46 ± 0.08 (7)

[1] Values are means ± SEM (*n*). Means in a column with superscripts without a common letter differ, $P < 0.05$. Lycopene concentrations less than the detection limit of 0.0037 nmol/g were entered as zero; thus, a mean of 0 ± 0 means that the supplement was not detected in any of the mice. The concentrations of lycopene and α- and γ,δ-tocopherol in all mice were greater in liver than in prostate.

tively, into liver and prostate reached physiologic concentrations that might explain the antitumor action in the target tissue. Prostate lycopene concentrations (0.014–0.038 nmol/g) were roughly comparable to those in ferrets (53), but liver lycopene concentrations (0.07–0.32 nmol/g) were lower than reported for other species, especially rats (13,53,54). The lower liver lycopene concentrations might relate to both the generally low bioavailability of carotenoids in mice and the administration of lycopene through oral gavage without the use of oil. In our study, α-tocopherol levels in mice daily gavaged with 50 mg vitamin E/kg BW were about half of those reported for rat liver (55) and prostate (46) in studies in which 50–540 mg vitamin E was incorporated into the diet.

Consistent with previous observations in humans (56) and rodents (53,54), *cis*-isomers accounted for the majority of tissue lycopene, although all-*trans* lycopene was the predominant form in the supplement. The largest percentage of the 5-*cis* isomer was found in the prostate, and this was further enhanced as lycopene intake increased. A preferential increase of 5-*cis* lycopene with increasing doses of lycopene also occurred in rats (54). The biological relevance of the specific lycopene isomers remains to be defined.

The mechanisms through which the lycopene-vitamin E mix may have inhibited tumor growth remain speculative. Both nutrients can influence a variety of biologic processes by mechanisms dependently and independently of their antioxidant functions (12–15,57). They may lower oxidative stress (12,13,58) and affect cell cycle progression (12,59–63), hormone and growth factor signaling (46,64,65), cell communication (66,67), and apoptosis (61,68–70). Cooperative interaction between lycopene and vitamin E (32,46,49) might result from a different mechanism of action or a direct effect of the nutrients on each other, e.g., by preventing oxidation (49,71,72) or cleavage (73), or by altering pharmacodynamics (33,74).

Although large-scale phase III/IV trials are eventually needed for proof of clinical benefit, they are not suited for initial screening, dose-finding, and multiagent testing. It might be more feasible to explore and optimize potentially interesting phytochemicals and their combinations in preclinical models first, followed by careful testing in Phase II trials to assess effects

TABLE 3

The percentage of total lycopene present as all-trans, 5-cis or other cis-lycopene isomers in prostate and liver tissues of mice supplemented orally with varying doses of lycopene and/or vitamin E

Tissue	Daily oral treatment (mg/kg BW)	*n*[1]	All-*trans* lycopene[2]	5-*cis* lycopene	Other *cis*-lycopene isomers
			%		
Prostate	Lycopene (5)	5	29.5 ± 1.6[a]	53.7 ± 2.7[a]	16.8 ± 1.4
	Lycopene (50)	4	22.7 ± 1.8[b]	62.8 ± 0.6[b]	14.6 ± 2.1
	Lycopene + Vitamin E (5 each)	7	32.6 ± 1.4[a]	55.4 ± 1.6[a]	12.1 ± 2.2
Liver	Lycopene (5)	9	33.1 ± 1.5	43.1 ± 1.3	23.7 ± 1.5
	Lycopene (50)	8	32.1 ± 5.9	44.7 ± 4.1	23.3 ± 2.1
	Lycopene + Vitamin E (5 each)	7	28.3 ± 1.0	44.2 ± 1.3	27.5 ± 1.6
LycoVit[3]		NA	77	22	1–2

[1] Numbers of samples analyzed.
[2] Values are means ± SEM (*n*). For a given tissue, means in a column with superscripts without a common letter differ, $P < 0.05$. Overall, prostate contained significantly more 5-*cis* lycopene than liver, but fewer other *cis*-lycopene-isomers.
[3] For comparison, the lycopene isomer composition of Lycovit is shown as well. NA, not applicable.

upon surrogate endpoints (30,75,76). PSA may be a useful marker in these preliminary prostate cancer trials, provided selective effects of candidate compounds on PSA are excluded (29,30, 76). In the present study, PSA:tumor ratios did not differ among the groups, indicating that lycopene and vitamin E do not selectively affect PSA. These data give support to the thesis that the lowering of PSA observed in short-term lycopene intervention studies relates to an effect on tumor growth (26–28).

In conclusion, this study demonstrates a benefit of lycopene plus vitamin E in reducing prostate cancer growth and corroborates PSA as a marker for tumor burden for this treatment regimen. On the basis of our findings and the available evidence (6,26,27,32), we are currently assessing the effects of lycopene-vitamin E supplementation on rising PSA-levels in an exploratory phase II clinical prostate cancer trial.

ACKNOWLEDGMENTS

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