Lycopen, a Dietary Cancer Chemopreventive Agent

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1. INTRODUCTION

Lycopen (Fig. 1), β-carotene, and lutein are the three most abundant carotenoids in the human diet (1). Of more than 600 natural carotenoids, lycopen is the most efficient singlet oxygen quencher, and is therefore a potent antioxidant (2,3). Although twice as efficient as β-carotene at quenching singlet oxygen (3,4), lycopen has no provitamin A activity, so its potential pharmacological effects cannot be associated with vitamin A. Lycopen is found in food primarily as the all-trans isomer, the form that is biosynthesized by plants and found in tomatoes, watermelon, and pink grapefruit (5). However, when exposed to heat and light during cooking or food processing, lycopen will produce a variable mixture of all-trans-lycopenes and cis isomers.

Reviews by Gerster (6) and van Poppel (7) examined the findings of more than 77 retrospective dietary studies and 55 prospective dietary or blood-level studies investigating the anticarcinogenic effects of carotenoids. These data overwhelmingly support the hypothesis that a diet rich in fresh fruits and vegetables reduces the incidence of cancer at certain sites. The overriding question posed by these reviews is, “What are the active anticancer agents in these diets?” Hydrocarbon carotenes and oxygenated xanthophylls comprise the highly conjugated class of pigments known as carotenoids (8).

Many key biological roles are ascribed to various carotenoids in both the plant (9) and animal kingdoms (10). In addition, a growing body of evidence acquired over the past decade suggests that carotenoids may function to prevent and/or attenuate certain deleterious human health conditions (11). In most dietary carotenoid studies to date, β-carotene intake and/or blood levels were measured and correlated with cancer prevention. However, few epidemiological studies have included measurements of the blood or tissue levels of other carotenoids. Furthermore, because of analytical limitations, the amounts of cis- vs all-trans carotenoid isomers could not be examined until most recently. For example, the separation of all-trans-lycopenes from various cis isomers using current high-pressure liquid chromatography (HPLC) technology and on-line mass spectrometric detection is shown in Fig. 2. Synthetic β-carotene used for dietary supplementation is all-trans. Fresh root vegetables such as carrots and sweet potatoes contain exclusively all-trans carotenoids (12). Green leaves, vegetables exposed to sunlight, and cooked vegetables including cooked carrots and sweet potatoes have significant levels of cis carotenoids (12).

Since lycopen is such a potent antioxidant, its anticancer activity might be the result of chemoprotection from oxidative stress that results from an imbalance in the prooxidant/antioxidant ratio in favor of the oxidants (13,14). The most important oxidants responsible for oxidative stress are free radicals and other reactive oxygen species (ROS), which can be formed as a result of exposure to toxic agents such as chemotherapeutic drugs or cigarette smoke (oxidants or prooxidants) or by inadequate dietary supplies of antioxidants (15).
Oxidative stress might contribute to aging, arteriosclerosis, rheumatoid arthritis, and cancer (15). Several reviewers have presented strong evidence that ROS play an important role in the development of cancer (15,16).

A great deal of evidence supports the link between oxidative stress and cancer. Clinical trials using antioxidants (i.e., the Linxian, China studies (17) and numerous case-control investigations) demonstrated that cancer prevention was associated with higher levels of plasma nutrient antioxidants (18,19). More than 200 cancer studies with animal models demonstrated a protective effect (at the levels of both initiation and promotion) of nutrient antioxidants and a large variety of nonnutritive antioxidants (20–22). An increased risk of cancer has been found in conditions that produce ROS, such as chronic inflammation (23). Free radicals are known to mediate the activation of carcinogens, which can be prevented by the addition of antioxidants (24). Cellular prooxidative states that result in increases in ROS, such as exposure to organic peroxides and the hydroxy radical, can promote initiated cells to neoplastic growth (25). And finally, many tumor promoters are prooxidants, or produce prooxidative states (26).

This chapter summarizes the increasing clinical and preclinical evidence suggesting that lycopene might serve as a chemopreventive agent in certain forms of cancer.

2. CLINICAL STUDIES OF LYCOPENE

Numerous epidemiological studies have investigated which components of dietary fruits and vegetables are most responsible for their cancer preventive effects. Overall, these studies suggest that dietary intake of carotenoids is inversely correlated with the incidence of many forms of cancers, and lycopene in particular is indicated for prevention of certain types of cancer (27–31). For example, Cramer et al. (32) carried out a dietary questionnaire-based study of 549 women with ovarian cancer and 516 control subjects. They found that the consumption of lycopene, especially in the form of tomato sauce, was significantly and inversely associated with the risk of ovarian cancer in premenopausal women. In contrast, α-carotene and carrot consumption were more significant for reducing the risk of ovarian cancer in postmenopausal women. To date, no reports of prospective epidemiological studies or lycopene intervention trials have confirmed these findings regarding ovarian cancer chemoprevention by lycopene.

In a study by Helzlsouer and colleagues (33), lycopene was suggested to reduce the risk of bladder cancer. The objective of the study was to examine the association between serum nutrients and the development of bladder cancer. Lycopene, selenium, α-tocopherol, β-carotene, and retinol were measured in serum collected from more than 25,000 persons and kept frozen for 12 yr. During that period, 35 cases of bladder cancer developed among participants. Nutrient serum levels among the cancer cases were compared to those of two matched controls for each case. It was found that lycopene and selenium were lower among the cancer cases, suggesting that these two compounds might provide protection against bladder cancer. In a related prospective study of this same study population by Sato et al. (34), 295 cases of breast cancer and 295 matched controls were evaluated, and dietary lycopene was among the carotenoids associated with a reduced risk of breast cancer.

In a pilot study of 32 women, Kanetsky (35) used dietary recall interviews and noted that women in the upper levels of lycopene intake were one-fourth as likely to suffer from cervical dysplasia as women in the lower intake groups. Cervical dysplasia is a risk factor for developing cervical carcinoma. In a similar study, Goodman et al. (36) reported on 238 women, 147 with dysplasia and 191 controls, and found that mean levels of lycopene were significantly lower among women with cervical dysplasia than in control subjects. Furthermore, inverse dose-response trends were observed for the carotenoids lycopene and α-cryptoxanthin and the antioxidants vitamin C and α-tocopherol. To the best of our knowledge, no clinical intervention studies have evaluated the chemopreventive activity of lycopene in digestive cancer, bladder cancer, cervical dysplasia, and cancer of the cervix.
By far the most compelling evidence so far in support of lycopene as a cancer chemoprevention agent has been in studies of men with prostate cancer, the second leading cause of cancer death among American men over age 65. In 1995, Giovannucci et al. (37) reported the results of a single dietary assessment with follow-up of 51,529 male health professionals; 773 of these developed prostate cancer over a 6-yr period. Higher intake of lycopene and tomato products was found to be associated with lower risk of prostate cancer. Giovannucci (38) reaffirmed these results in a review of the epidemiological evidence available from multiple sources. Recently, Gann et al. (39) and Giovannucci et al. (40) reported results for a prospective study of this same cohort of men with multiple dietary assessments during a period of 12 yr. During this extended period of time, 2481 cases of prostate cancer occurred in the study group. These prospective epidemiological analyses confirmed the inverse correlation between prostate cancer and the consumption of tomato products.

Coincident with the epidemiological studies of lycopene and prostate cancer reported by Giovannucci and colleagues, Clinton et al. (41) measured lycopene in a variety of human tissues, finding that lycopene and particularly its cis isomers, are selectively concentrated in the human prostate. These results suggest that lycopene might be an active component of the tomato that serves as a prostate cancer chemopreventive agent. In support of this hypothesis, prospective and retrospective epidemiological studies reported by Giovannucci et al. showed a significantly lower risk of prostate cancer in men who consumed tomato products that were rich in lycopene. These statistically significant findings all suggest that lycopene shows promise as a cancer chemopreventive agent in the human prostate.

In addition to these epidemiological studies, a four-arm Phase II clinical trial in progress, under the direction of van Breemen, is investigating the efficacy of lycopene as a prostate cancer chemopreventive agent. In this study, lycopene is administered as a tomato oleoresin at 30 mg/d and measured in the serum of men before and after a 21-d intervention period. Lycopene is also being measured in prostate tissue biopsy specimens. DNA oxidation products, including 8-oxodeoxyguanosine (8-oxo-dG), 5-hydroxymethyluridine, and 8-oxo-deoxyadenosine (8-oxo-dA) are being measured in peripheral-blood leukocytes before and after lycopene intervention. These DNA modifications are also measured in prostate tissue taken from biopsy specimens and are evaluated as intermediate endpoints for preventing DNA oxidation by lycopene. In addition, prostate-specific antigen (PSA) is measured in blood before and after the intervention period.

Intervention with tomato sauce was evaluated in a completed unblinded fifth arm of this study (42),
Among the 32 men who completed this preliminary dietary study, 75% were African-American, an ethnic group at particularly high risk for prostate cancer. Each patient served as his own control, since blood and prostate tissue biopsies were obtained at the start of the intervention period and blood and prostate tissue (from prostatectomy) were obtained after the 21-d dietary intervention period. Serum and prostate lycopene levels increased two- and threefold, respectively, following administration of 30 mg/d of dietary, tomato-based lycopene. The results of these assays are summarized in Table 1. Although these results indicate that an imperfect correlation exists between serum lycopene and prostate levels, they confirm studies by other groups showing that oral administration of lycopene or food rich in lycopene results in elevated serum levels (43,44). These data also confirm the observations of other groups who have found a trend between serum and prostate levels of lycopene in men who are undergoing prostate surgery (45). However, the bioavailability of lycopene and the amount of an oral dose that reaches the prostate could not be determined from these studies; other studies should be designed to elicit this information.

In addition to measuring total lycopene levels in prostate tissue and serum from these men who consumed dietary, tomato-based lycopene, van Breemen and colleagues (46) used liquid chromatography-mass spectrometry (LC-MS) with C30 reversed-phase chromatography and atmospheric pressure chemical ionization (APCI) to measure the ratio of cis/trans-lycopene in these serum and prostate tissue extracts (see example in Fig. 2). This analytical method facilitated the detection of all-trans-lycopene and up to 14 cis-isomer peaks in a single LC-MS chromatogram with a limit of detection of 0.93 pmol. They found that serum contained approx 28.9% all-trans-lycopene before dietary intervention and 31.7% all-trans-lycopene after intervention, despite the fact that the administered form of lycopene was 83% all-trans. This percentage of all-trans-lycopene is essentially identical to the 30.6% value measured in a solution containing a thermodynamic mixture of lycopene isomers, suggesting that lycopene rapidly isomerizes in serum just as it does in solution. In contrast, the percentage of all-trans-lycopene in human prostate tissue (lower than that in human serum) increased from 12.4–22.7% as a result of dietary intervention with tomato sauce.

A comparable observation was reported earlier by Clinton et al., who found that in the human prostate, all-trans-lycopene constituted 12–21% of the total (41). These studies showed that cis-lycopene accumulates preferentially in the human prostate and the proportion of cis-lycopene in human prostate tissue is greater than that in human serum. The mechanism for this accumulation and the reasons for different ratios of cis and all-trans isomers in prostate compared to serum remain unknown. Our data are consistent with the hypothesis that all-trans-lycopene is taken up preferentially by the prostate and then isomerized to cis forms. Other explanations are possible, including selective absorption of cis-lycopene isomers by the prostate, selective stabilization of cis isomers, and selective degradation of all-trans-lycopene in the prostate. The correct explanation for these results requires additional investigation.

Finally, total PSA levels were measured in blood samples at baseline, and again at completion of the
tomato sauce intervention period in order to evaluate lycopene's possible effect on PSA levels (results shown in Table 1). This dietary intervention study found that levels of PSA decreased by a statistically significant 17.5%. These results were confirmed in a pilot study reported by Kucuk et al. (28) in which 15 men with prostate cancer received 30 mg lycopene/d for 21 d following the same design as that of the van Bremen study. However, the study by Kucuk lacked sufficient power to be statistically significant.

PSA is produced by the epithelial cells of the prostate and secreted into the seminal fluid (47). Compared to the concentration of PSA in seminal fluid, the concentration of PSA in blood is small. Increased levels of PSA in blood are not the result of greater production of PSA by cells, but of abnormalities in the prostate gland architecture, which can be caused by trauma or disease (48). Because of its relative tissue specificity, serum PSA is used in combination with the digital rectal examination to screen men for prostate cancer. Currently, biopsy is recommended in men with PSA levels that exceed 4 ng/mL. Cancer is detected in approx 25% of men with PSA values between 4 and 10 ng/mL; more than one-half of men with PSA values exceeding 10 ng/mL have advanced cancer (49). PSA is also used to monitor the success of radiotherapy, chemotherapy, and surgery for the treatment of prostate cancer (50). Given the association of high serum PSA levels with prostate cancer, it is a significant finding that administration of 30 mg/d of lycopene for just 21 d reduced serum PSA levels in men with prostate cancer. Whether this indicates that lycopene might have value as a chemotherapeutic agent for prostate cancer, in addition to efficacy as a chemopreventive agent, has not yet been determined.

3. ANIMAL MODELS AND STUDIES OF LYCOPENE CANCER PREVENTION

Although the consumption of lycopene-containing tomatoes has been clearly associated with a reduced risk of prostate cancer, these epidemiological results have not been reproduced in animal models of prostate cancer. In male F344 rats, ventral prostate cancer was induced with 3,2'-dimethyl-4-aminoazobenzene (DMAB) and 2-amino-1-methylimidazo[4,5-b]pyridine (PhIP). Dietary lycopene of up to 45 mg/kg failed to prevent DMAB-induced ventral prostate carcinomas (51). In the prostate of lacZ mice, both benzo[a]pyrene (B[a]P)-induced and spontaneous mutagenesis were only slightly inhibited by oleoresin (52). The uptake and tissue distribution of lycopene in F344 rats fed a diet supplemented with 10 mg/kg of lycopene for 2 mo was compared to that of humans. In rats, lycopene concentration was determined to be highest in the spleen (21.2 nmol/g) and liver (20.3 nmol/g), followed by the prostate (0.3 nmol/g) and lung (0.1 nmol/g) (30). Interestingly, the human prostate accumulates higher lycopene levels (0.8 nmol/g) than the rat prostate (30). It is unlikely that the difference in tissue levels is solely responsible for the failure of lycopene to prevent tumors in the rat prostate. There are several possible explanations for the failure of animal prostate cancer models to reproduce human epidemiological data. Animal models of prostate cancer may be flawed because they use large doses of carcinogens that do not mimic the human situation. Lycopene is metabolized differently in the animal prostate because of anatomical differences between human and rat or mouse prostate. Lycopene does not reach effective concentrations in the rat prostate. And, finally, animal studies that use purified lycopene (or the tomato extract oleoresin) do not derive the benefit of synergism between different food components.

Animal models of breast cancer have revealed mixed results. Young female Sprague-Dawley rats treated with 7,12-dimethylbenz[a]anthracene (DMBA) or N-methyl-N-nitrosourea (MNU) develop mammary tumors. DMBA requires metabolic activation, whereas MNU is a direct-acting carcinogen. Intraperitoneal administration of lycopene-enriched tomato oleoresin suppressed DMBA-induced mammary tumor development in rats (53). In this study, rats were injected with oleoresin (10 mg/kg, twice per wk) for 2 wk prior to tumor induction by DMBA and for an additional 16 wk after carcinogen administration. Oleoresin-treated rats developed significantly fewer tumors, and tumor volume was smaller than that of the unsupplemented rats. In contrast, β-carotene was found to be ineffective (53).

Dietary oleoresin and pure lycopene were evaluated in the MNU-induced mammary tumorigenesis model in female Sprague-Dawley rats (54). Rats were fed diets supplemented with 250 and 500 mg/kg lycopene, or oleoresin providing equivalent amounts of lycopene. The rats began eating the experimental or control diets 1 wk before initiation with MNU and continued for an additional 18 wk. Pure lycopene and oleoresin did not exert an inhibitory effect on tumor incidence, latency, multiplicity, volume, or total tumors per group compared with unsupplemented controls. In this study,
total lycopene concentration of about 0.16 μM was measured in the serum of rats fed the high dose of oleoresin. Administration of lycopene to female rats at doses ranging from 0.001–0.1 g/kg bw per d for 2 wk resulted in lycopene plasma concentration ranging from 0.016 μM at the low dose to 0.067 μM at the high dose (55). According to Stahl and Sies (56), lycopene concentrations in rats are much lower than those reported in human populations, where the plasma concentration of lycopene ranges from 0.22 to 1.06 μM or from 0.07 to 1.790 μM, according to Breinholt and colleagues (55). For example, men who consumed a tomato-rich diet had a mean serum lycopene concentration of 1.3 μM (46). Elderly women who consume diets rich in fruits and vegetables had a plasma mean concentration of total lycopene of 0.43 μM (the concentration of lycopene in younger women or younger or older men was higher) (57). Conflicting results in the two models of chemoprevention might be the result of differences in the two carcinogens, one being a direct-acting carcinogen (MNU) and the other (DMBA) requiring activation by the host. Differences in the route of lycopene administration further complicate comparisons between the two studies. In the Cohen et al. study (54), lycopene or oleoresin incorporated into the diet mimicked the human situation. In contrast, the intraperitoneal administration of lycopene in the study of Sharoni et al. (53) circumvented the process of digestion and absorption into lymphatic chylomicrons.

Through its action on cytochrome P450, lycopene may diminish the effects of carcinogens requiring metabolic activation such as DMBA. Breinholt et al. (55) determined the drug metabolizing capacity and antioxidant status of female rats exposed to lycopene diets. They found that the activities of the liver cytochrome P450-dependent enzymes, benzoylxyresorufin O-dealkylase and ethoxyresorufin O-dealkylase, were significantly induced by lycopene administration. Also, the phase II detoxification enzymes, glutathione S-transferase (GST) and quinone reductase, were induced by lycopene in the liver. Superoxide dismutase (SOD), glutathione reductase, and glutathione peroxidase in the blood were also induced by the administration of lycopene. These studies suggest that modulation of antioxidant and drug metabolizing enzymes might indeed be stronger in humans whose plasma lycopene levels are several-fold higher than those reported in rats (55).

An investigation by Kim et al. evaluated the chemopreventive potential of lycopene during the post-initiation stage in a multi-organ carcinogenesis model (58). B6C3F1 mice, of both sexes were subjected to treatment with a combination of three carcinogens, diethylaminoethyl nitrosamine (DEN), MNU, and 1,2-dimethylhydrazine (DMH), for up to 9 wk after birth. Lycopene at 25 and 50 mg/kg diet was given after wk 11. Aberrant crypt foci (ACF) and tumors in the colon, kidney, liver, and lung were determined. The incidence and multiplicity of lung adenomas plus carcinomas were significantly reduced only in the group of male mice that received 50 mg/kg-diet lycopene. No such effect was observed for females. Although the effect of lycopene on hepatocellular carcinomas was inconclusive, the carotenoid was ineffective in preventing colon and kidney tumors in this study. The results suggest that lycopene exerts a chemopreventive effect limited to male lung carcinogenesis when given in the post-initiation stage to B6C3F1 mice. In A/J mice, oleoresin (up to 8.3 g/kg diet) was ineffective against B[a]P plus 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorogenesis (59).

Inhibition of colon carcinogenesis by lycopene and tomato juice was investigated in young female F344 rats (60). Rats received MNU, and had free access to plain drinking water containing lycopene, or water containing diluted tomato juice. The incidence of colon cancer was significantly lower in the group that received tomato juice but not the group that received lycopene (60). These results show that tomato juice furnishes protection against colon carcinogenesis, and suggest that lycopene and other components of oleoresin might act synergistically. In a related study, the effects of oleoresin on long-term (spontaneous) carcinogenesis were compared to B[a]P-induced short-term effects in lacZ mice (52). Oleoresin inhibited spontaneous but not B[a]P-induced mutagenesis in the prostate and the colon. This study suggests that the antioxidant action of oleoresin might neutralize low levels of pro-oxidants that occur during normal metabolism, but not high levels that are introduced by carcinogens in short time intervals.

Based on the observation that patients with hepatitis and cirrhosis have low plasma lycopene levels (61), the effects of lycopene on occurrence of hepatic neoplasia were evaluated in rats. Lycopene-containing oleoresin was added to the diet at 5 g/kg. It offered no protection against spontaneous hepatocarcinogenesis or enhanced survival in rats (62). Although oxygen radicals play an important role in the development of hepatitis and subsequent liver cancer, it appears that
the antioxidative activities of lycopene might be insufficient to prevent hepatocarcinogenesis in the rat. However, hepatic fibrogenesis was suppressed in a strain of rats that consumed lycopene in their diet (63). The mechanism of this preventive effect was through inhibition of stellate-cell activity. Dietary lycopene has been shown to decrease the initiation of liver carcinomas in male C3H/He mice after combined treatment with 4-nitroquinoline-1-oxide and glycerol (64). In rats, oxidative stress introduced by ferric nitrolotriacetate (Fe-NTA) was determined to cause increased 8-oxo-dG levels in the liver accompanied by histopathological changes (65). Lycopene (10 mg/kg body wt) almost completely prevented this oxidative damage and protected the liver against observed histological alterations.

Tomato juice exerts an inhibitory effect on development of urinary bladder carcinoma induced by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in rats (66). The carcinoma was administered in the drinking water of male F344 rats for 8 wk followed by diluted tomato juice for 12 wk. In the group that was given tomato juice, the numbers—but not the incidence of—urinary bladder transitional cell carcinomas were decreased. Nodulopapillary hyperplasias, invasion, or differentiation of transitional cell carcinomas were not affected by this treatment (66). In the same animal model, piroxicam—a nonsteroidal antiinflammatory drug (NSAID)—in combination with lycopene significantly decreased the incidence and number of transitional cell carcinomas (67).

Recently, Boileau et al. (68) reported that cis isomers of lycopene were more bioavailable than all-trans-lycopene in the ferret. This information, in combination with measurements showing that cis-lycopene isomers often constitute more than 50% of total lycopene in human blood and tissues (41,69), has prompted speculation that cis-lycopenes might be more readily absorbed by humans. However, data on the human bioavailability of lycopene and its isomers are scarce. Since human tissue specimens are often limited to biopsies less than 10 mg, a reliable and sensitive method is needed to simultaneously measure cis- and trans-lycopene in these samples. Recently, van Breemen et al. (46) developed a highly sensitive and specific LC-MS method to address this problem and applied it to the analysis of lycopene isomers in human serum and prostate tissue of men with prostate cancer before and after lycopene intervention. An example of this analysis is presented in Fig. 2. This new analytical capability might allow scientists to determine if cis-lycopene isomers are more bioavailable than trans-lycopene.

4. CELL-BASED STUDIES OF LYCOPENE

Lycopene has been found to inhibit the proliferation of several types of cancer cells, including those of prostate, breast, endometrium, leukemia, and lung (70–76). Although the precise mode of action is not fully understood, several molecular pathways have been proposed.

Lycopene, as well as other acyclic carotenoids such as phytoflueine and ζ-carotene, present in the tomato, significantly reduced the viability of PC-3, DU-145 and LNCaP human prostate cancer cell lines (70). However, lycopene tended to reduce cell viability at a lower concentration than did the other acyclic carotenoids. Generally, 5 μM lycopene reduced cell viability to about the same magnitude as 20 μM of phytoflueine and ζ-carotene. In a previous study, Pastori et al. reported that lycopene at 1.8 μM inhibited the proliferation of PC-3 and DU-145 cells when given in combination with 50 μM α-tocopherol (71). These concentrations, considered physiologically relevant, inhibited by almost 90% the growth of both types of prostate cancer cells. The synergism between lycopene and α-tocopherol was not shared by β-tocopherol, ascorbic acid, and probucol (71).

Karas et al. (72) have shown that, in MCF-7 breast cancer cells, growth stimulation by insulin-like growth factor I (IGF-I) was reduced by physiological concentrations of lycopene. IGF-I acts as a mitogen, and is considered a risk factor in breast and prostate cancer. The inhibitory effect of lycopene on IGF signaling was associated with suppression of cell cycle progression without being associated with apoptotic or necrotic cell death. A delay in the progression of MCF-7 cells from G1 to S was also observed in response to lycopene or acycloretinoic acid treatments (73). Since the concentrations of acycloretinoic acid and lycopene required for inducing cell growth inhibition were similar, it was concluded that acycloretinoic acid is unlikely to be the active metabolite of lycopene. Based on the low affinity of lycopene for retinoic acid receptor (RAR) and a low efficacy in activating the receptor, it was also determined that RAR does not mediate the growth-inhibitory effect of the agent. Since lycopene inhibited growth of both the estrogen receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 cells, it was
concluded that ER is not involved in the inhibition of breast cancer cells (74).

Nahum et al. dissected the mechanism by which lycopene (2–3 μM) delays cell cycle in synchronized human breast (MCF-7, T47D) and endometrial (ECU-1) cancer cells (75). In these cells, inhibition of growth and block in cell cycle progression at the G1 phase was associated with reduced cyclin-dependent kinase (CDK)4 and CDK2 activities. A decrease was also evident in cyclin D1 and cyclin D2 levels that was not accompanied by reduced cyclin E levels. Cyclin D1 is a known oncogene, and its overexpression is associated with malignancy. Based on alterations in the levels of CDK inhibitors p21(Cip1/Waf1) and p27(Kip1) in lycopene-treated (cell cycle-arrested) or serum-stimulated cells, the following chain of events was proposed to explain the inhibitory effect of lycopene on cell cycle progression. Retention of p27 in the cyclin E-CDK2 complex causes a decrease in cyclin D, which leads to reduction in CDK4 kinase activity and subsequent decrease in pRb phosphorylation, resulting in inhibition of G1/S transition (75).

In human fetal skin fibroblasts (HFF02 cells), lycopene at a concentration of 0.1 μM was found to be an effective inducer of gap-junctional communication. This was determined by microinjecting fluorescent Lucifer Yellow CH dye into selected HFF02 cells in a confluent culture that had been treated or not with lycopene, and then measuring the transfer of fluorescence to adjacent cells 5 min later (77). The lycopene oxidation product acyloretinoid acid also increased gap-junctional communication but required a 10-fold higher concentration for the same effect. Induction of gap-junctional communication, unlike that of retinoic acid and other carotenoids, was not mediated by stabilizing the mRNA of connexin43 (78). Induction of gap-junctional communication in the lycopene-treated cells was suggested to be related to cancer prevention (79).

In the HL-60 promyelocytic leukemia cell line, a concentration-dependent reduction in HL-60 cell growth was shown to be accompanied by cell differentiation and delay in the G1/G2 phase of the cell cycle (76). More importantly, lycopene was shown to act synergistically with 1,25-dihydroxyvitamin D3 in both inhibiting HL-60-cell proliferation and inducing the differentiation of these cancer cells (76). These results may suggest that dietary carotenoids may act as chemopreventive agents only in conjunction with other dietary components, providing a possible explanation for the failure of human intervention studies in which β-carotene was used only in conjunction with either α-tocopherol (80,81) or with retinyl palmitate (82).

Matos et al. (83) investigated the antioxidant effects of lycopene against iron-induced oxidative stress, using Fe-NTA, known to induce lipid peroxidation, DNA damage, and renal carcinomas in rats. The effect of lycopene on lipid peroxidation and on formation of 8-oxo-dG was determined in green monkey kidney fibroblasts (CV1-P) exposed to Fe-NTA plus ascorbate. Lycopene produced an 86% reduction in Fe-NTA/ascorbate-induced lipid peroxidation, which was associated with a substantial decrease in 8-oxo-dG levels. These results indicate that lycopene, by protecting mammalian cells against membrane and DNA damage, might prevent tumor promotion associated with oxidative stress.

In an interesting study, lycopene was entrapped in human albumin and its effect against oxidative 1O2 attack was determined by detecting 8-oxo-dG and 4-hydroxy-8-oxo-dG. The lycopene/albumin complex reduced oxidative DNA damage by 50–70% compared to the control. This experiment suggested that lycopene entrapped in albumin can be an efficient quencher of 1O2 and in this manner may provide protection against the deleterious effect of this ROS (84). Furthermore, lycopene has been shown to exhibit much more potent antiproliferative effects than either α- or β-carotene against endometrial (Ishikawa), mammary (MCF-7), and lung (NCI-H226) human cancer cells, with half-maximal inhibitory concentrations of 1–2 μM (85). In comparison, α- or β-carotene required from four- to 10-fold higher concentrations to exert the same antiproliferative effect as lycopene.

5. SUMMARY AND CONCLUSIONS

A large number of prospective and retrospective epidemiological studies suggest an inverse relationship between prostate cancer and the consumption of tomato products. In men, the consumption of tomato products is associated with increased levels of lycopene in serum and especially in the prostate gland. The results of the first human intervention studies with tomato sauce show a marked increase in lycopene levels in the prostate 3 wk after the intervention, accompanied by a decrease in PSA levels and a decrease in oxidative DNA damage (42). The tissue concentration of lycopene as well as the chemical form might be important for chemopreventive efficacy; emerging studies
suggest that the cis isomer found in prostate and liver, especially after castration, might be the effective form (68, 86). Consumption of tomato products has also been linked to a reduced risk for ovarian cancer, urinary bladder cancer, breast cancer, cancer of the digestive system, and cancer of the cervix. However, the evidence that tomato products or lycopene might be beneficial for organs other than the prostate is slim, based mainly on dietary recall interview studies and cell culture studies.

Studies in rat models of prostate cancer chemoprevention have generally failed to show the expected outcome. This might be caused by either the high doses of carcinogens that are routinely used in these models or the bioavailability and tissue distribution of lycopene in the rat prostate, which seems to be different from that of the human prostate. Most animal studies have also shown that lycopene and oleoresin are generally ineffective in preventing chemically induced colon, lung, and liver cancer. Lycopene seems to provide better protection against spontaneous tumors that (unlike induced tumors) do not require exposure to high doses of carcinogens—a quality consistent with its ability to quench free radicals. Studies in cultured cells showed clearly that lycopene can prevent certain types of oxidative DNA damage at physiologically relevant concentrations, but also revealed other possible mechanisms of action. These include an antiproliferative effect caused by cell cycle arrest, induction of differentiation, and stimulation of gap-junctional communication (73, 75, 76). Studies in cultured cells also revealed that lycopene might be more effective as an antioxidant agent when given in combination with other agents (e.g., vitamin D3, or α-tocopherol) (71, 76). This latter observation provides a possible explanation of why animal studies generally show that lycopene is ineffective, and human studies suggest the opposite. In humans that eat a variety of foods, synergism between lycopene and other dietary components is possible, yet animals fed purified lycopene or oleoresin have little chance for synergism unless the experiment is designed to evaluate that question.

In conclusion, efficacy of lycopene may depend on its tissue distribution, bioavailability, and the presence of other dietary components that may work together in a synergistic manner. The mechanisms of lycopene action involve protection against oxidative DNA damage and membrane damage either directly through chemical quenching or indirectly through the activation of phase II detoxification enzymes. Further studies are needed in all levels (in vitro, cell culture, animal models of cancer, and clinical intervention) to elucidate the precise mechanism of action and to determine the chemopreventive efficacy of this promising carotenoid. Particular emphasis should be given to a possible synergism between cis-lycopene isomers and other food components, especially other antioxidants.

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