Overview of Mechanisms of Action of Lycopene

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Dietary intakes of tomatoes and tomato products containing lycopene have been shown to be associated with decreased risk of chronic diseases such as cancer and cardiovascular diseases in numerous studies. Serum and tissue lycopene levels have also been inversely related to the risk of lung and prostate cancers. Lycopene functions as a very potent antioxidant, and this is clearly a major important mechanism of lycopene action. In this regard, lycopene can trap singlet oxygen and reduce mutagenesis in the Ames test. However, evidence is accumulating for other mechanisms as well. Lycopene at physiological concentrations can inhibit human cancer cell growth by interfering with growth factor receptor signaling and cell cycle progression specifically in prostate cancer cells without evidence of toxic effects or apoptosis of cells. Studies using human and animal cells have identified a gene, connexin 43, whose expression is upregulated by lycopene and which allows direct intercellular gap junctional communication (GJC). GJC is deficient in many human tumors and its restoration or upregulation is associated with decreased proliferation. The combination of low concentrations of lycopene with 1,25-dihydroxyvitamin D3 exhibits a synergistic effect on cell proliferation and differentiation and an additive effect on cell cycle progression in the HL-60 promyelocytic leukemia cell line, suggesting some interaction at a nuclear or subcellular level. The combination of lycopene and lutein synergistically interact as antioxidants, and this may relate to specific positioning of different carotenoids in membranes. This review will focus on the growing body of evidence that carotenoids have unexpected biologic effects in experimental systems, some of which may contribute to their cancer preventive properties in models of carcinogenesis. Consideration of solubility *in vitro*, comparison with doses achieved in humans by dietary means, interactions with other phytochemicals, and other potential mechanisms such as stimulation of xenobiotic metabolism, inhibition of cholesterogenesis, modulation of cyclooxygenase pathways, and inhibition of inflammation will be considered. This review will point out areas for future research where more evidence is needed on the effects of lycopene on the etiology of chronic disease. Exp Biol Med 227:920–923, 2002

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Lycopene cyclase is an enzyme found in tomatoes that can convert lycopene to β-carotene by catalyzing the formation of two β-rings at each end of the linear carotene. The red color of tomatoes is due to the accumulation of lycopene resulting from a down-regulation of the lycopene cyclase gene (CrtL), which has been cloned from tomato (*Lycopersicon esculentum*). Transcriptional down-regulation of this gene conserved in evolution from the time of cyanogenic bacteria is the predominant mechanism leading to lycopene accumulation during the process of fruit ripening in tomatoes (1). Therefore, lycopene from tomato products necessarily occurs in combination with related phytochemicals, including phytoene and phytofluene. Nonetheless, most research on the preventive effects of dietary intake of tomato products have focused on lycopene.

Increased ingestion of tomatoes and tomato products containing lycopene has been shown to be associated with decreased risk of chronic diseases including cancer. For example, serum and tissue lycopene levels have been inversely related with prostate cancer risk (2–5). Current dietary recommendations emphasize increasing the daily consumption of fruits and vegetables from diverse sources such as citrus fruits, cruciferous vegetables, and green and yellow vegetables (5). Each of these classes of plant-derived foods may have unique phytochemicals that interact with the host to confer a preventive benefit by regulating enzymes important in metabolizing xenobiotics and carcinogens, by modulating nuclear receptors and cellular signaling of proliferation and apoptosis, and by acting indirectly through antioxidant actions that reduce proliferation and protect DNA from damage (6). Although there is significant evidence supporting the actions of lycopene as a potent antioxidant, there are a number of other potential mechanisms through which tomato products providing lycopene and other phytochemicals may reduce the risk for chronic diseases, including common forms of cancer and heart disease. This review will focus on the growing body of evidence that lycopene and related carotenoids have unexpected biologic effects in experimental systems, some of which may contribute to their observed preventive properties.

Antioxidant Activity

Oxidative stress is recognized as one of the major contributors of increased risk of cancer, and in chemical assays, lycopene is the most potent antioxidant among various common carotenoids (7). Lycopene can trap singlet oxygen and reduce mutagenesis in the Ames test. The antioxidant activity of carotenoids in multilamellar liposomes has been assayed by inhibition of formation of thiobarbituric acid-reactive substances (8). In this assay, lycopene has been

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demonstrated to be the most potent antioxidant with the ranking: lycopene > α-tocopherol > α-carotene > β-cryptoxanthin > zeaxanthin = β-carotene > lutein. Mixtures of carotenoids were more effective than the single compounds. This synergistic effect was most pronounced when lycopene or lutein was present. The superior protection of mixtures may be related to specific positioning of different carotenoids in membranes.

The antioxidant properties of tomato products providing dietary lycopene including tomato juice, spaghetti sauce, and tomato oleoresin were studied in 19 healthy human volunteers using a randomized, crossover design in which each source of lycopene was administered for 1 week. Blood samples were collected at the end of each treatment. Serum lycopene was extracted and measured by high-performance liquid chromatography (HPLC) using an absorbance detector. Serum thiobarbituric acid-reactive substances, protein thiols, and 8-oxodeoxyguanosine contents of lymphocyte DNA were assayed to measure lipid, protein, and DNA oxidation. Lycopene was the major carotenoid present in the serum. Dietary supplementation of lycopene resulted in a significant increase in serum lycopene level and diminished amounts of serum thiobarbituric acid-reactive substances. Although not statistically significant, a tendency of lowered protein and DNA oxidation was observed. There was also indication that the lycopene levels increased in a dose-dependent manner in the case of spaghetti sauce and tomato oleoresin. These results indicate that lycopene absorbed from tomato products may act as an *in vivo* antioxidant (9).

Antiproliferative and Prodifferentiation Activities

Lycopene has been found to inhibit proliferation of several types of cancer cells, including those of breast, lung, and endometrium. The HL-60 promyelocytic leukemia cell line has been extensively studied with a variety of differentiating and antiproliferative agents. In studies by Amir *et al.* (10), lycopene resulted in a concentration-dependent reduction in HL-60 cell growth as measured by [^{3}H]thymidine incorporation and cell counting. This effect was accompanied by inhibition of cell cycle progression in the G0/G1 phase as measured by flow cytometry. Lycopene alone induced cell differentiation as measured by phorbol ester-dependent reduction of nitroblue tetrazolium and expression of the cell surface antigen CD14. The combination of low concentrations of lycopene with 1,25-dihydroxyvitamin D3 exhibited a synergistic effect on cell proliferation and differentiation and an additive effect on cell cycle progression. Such synergistic antiproliferative and differentiating effects of lycopene and other compounds found in the diet and in plasma suggest that phytochemicals such as vitamin D and lycopene, with separate modes of action, may unexpectedly combine to promote anticancer effects not seen with either agent alone. Because high doses of 1,25-dihydroxyvitamin D3 are required for antiproliferative effects and these doses are complicated by serious hypercalcemia, one solution may be to combine multiple compounds, including lycopene, in prevention efforts.

In another set of studies by the same group (11), the growth stimulation of MCF-7 mammary cancer cells by insulin-like growth factor 1(IGF-I) was markedly reduced by physiological concentrations of lycopene. The inhibitory effects of lycopene on MCF-7 cell growth were not accompanied by apoptotic or necrotic cell death, as determined by annexin V binding to plasma membrane and propidium iodide staining of nuclei in unfixed cells. Lycopene treatment markedly reduced the IGF-I stimulation of tyrosine phosphorylation of insulin receptor substrate 1 and the binding capacity of the AP-1 transcription complex. These effects were not associated with changes in the number or affinity of IGF-I receptors, but with an increase in membrane-associated IGF-binding proteins, which were previously shown in different cancer cells to negatively regulate IGF-I receptor activation. The inhibitory effect of lycopene on IGF signaling was associated with suppression of IGF-stimulated cell cycle progression of serum-starved, synchronized cells. Moreover, in cells synchronized by mimosine treatment, lycopene delayed cell cycle progression after release from the mimosine block. Collectively, the above data suggest that the inhibitory effects of lycopene on MCF-7 cell growth were not due to the toxicity of the carotenoid, but to interference in IGF-I receptor signaling and cell cycle progression. The significance of this finding for cancer prevention is related to independent epidemiological findings that elevated IGF-I levels increase lifetime risks of breast and prostate cancer. IGF-I is manufactured in the liver as the result of growth hormone stimulation, but circulating levels of IGF-I are modulated by nutritional status. Undernutrition is associated with reduced levels, whereas obesity in childhood is associated with markedly increased circulating levels of IGF-I. If lycopene interference with IGF-I stimulation of tumor cell growth is confirmed in clinical studies, this would provide a strong rationale for recommending increased intake of tomato products for cancer prevention.

Both carotenoids and retinoids stimulate gap junctional communication (GJC) through stabilization of connexin43 mRNA. Because GJC is lost in cancer cells, its restoration is considered to be a cancer-preventive property of carotenoids and retinoids. If lycopene is cleaved by analogy to the conversion of β-carotene to retinoic acid, then acyclo-retinoic acid is formed. Both lycopene and this cleavage product, which could result from oxidation, were tested for their effect on GJC, on stabilization of connexin43 mRNA, and on the transactivation of the RAR-β2-promoter *in vitro* by Stahl *et al.* (12). In human fetal skin fibroblasts, GJC was stimulated by lycopene and acyclo-retinoic acid. Lycopene was effective at a concentration of 0.1 μ*M*, whereas higher amounts of acyclo-retinoic acid (1 μ*M*) were needed for comparable stimulation. Stabilizing effects of acyclo-retinoic acid on the mRNA of connexin43 via elements located in the 3′-untranslated region were weak. In com-

parison with retinoic acid (0.1 μ*M*), considerably higher concentrations of the acyclo analog (50 μ*M*) were required for similar effects; lycopene (0.1 μ*M*) was not active in this system. Likewise, unphysiologically high levels of acyclo-retinoic acid (50 μ*M*) were necessary to transactivate the RAR-β2 promoter. The data demonstrate that acyclo-retinoic acid is much less active than retinoic acid with respect to GJC and retinoid-related signaling. These data are consistent with the conclusion that lycopene affects GJC independent of the formation of acyclo-retinoic acid. In fact, it is not established that acyclo-retinoic acid is an important physiologically active oxidation product of lycopene in humans. Therefore, although it is still possible that lycopene may act through the RAR receptor mechanism, much more research is needed on this question.

Hypocholesterolemic Effects

Both β-carotene and lycopene share similar initial synthetic pathways with cholesterol, which is synthesized in animal but not in plant cells. Fuhrman *et al.* (13) examined the effect of carotenoids on macrophage cholesterol metabolism in comparison with the effect of low-density lipoprotein (LDL) cholesterol and of the cholesterol synthesis inhibitor, fluvastatin. In J-774 A. 1 macrophage cell line, *de novo* cellular cholesterol synthesis from [^{3}H]acetate, but not from [^{14}C] mevalonate, was suppressed by 63% and by 73% following cell incubation with β-carotene or lycopene (10 μ*M*), respectively, in comparison with a 90% and 91% inhibition by LDL (100 μg of cholesterol) or by fluvastatin (10 μg/ml), respectively. However, unlike LDL-derived cholesterol, which also suppresses macrophage LDL receptor activity, lycopene and β-carotene augmented the activity of the macrophage LDL receptor, similar to the effect of fluvastatin. In agreement with these *in vitro* observations, dietary supplementation of lycopene (60 mg/day) to six men for a 3-month period resulted in a significant 14% reduction in their plasma LDL cholesterol concentrations. These findings suggest that dietary supplementation of carotenoids may act as moderate hypocholesterolemic agents, secondary to their inhibitory effect on macrophage 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) reductase, the rate-limiting enzyme in cholesterol synthesis. These observations have implications for both heart disease prevention through modification of the processes of cellular atherogenesis resulting in unstable plaque formation and on the process of carcinogenesis. Cancer cells have abnormal cholesterol biosynthetic pathways that are resistant to down-regulation by cholesterol, and farnesylation is a key process in oncogene activation (14). Supplementation with multiple carotenoids from fruits and vegetables, including tomato products, may help to prevent common forms of cancer.

Lycopene in Inflammation and Immune Function

In a cross-sectional study (15), the acute phase response of inflammation, as defined by an elevation of C-reactive protein, was associated with suppressed circulating levels of antioxidants in a population of 85 Catholic sisters (nuns) 77–99 years of age. The presence of an acute phase response was associated with an expected significant decrease in the serum concentrations of albumin ($P < 0.001$) and thyroxine-binding prealbumin ($P < 0.001$), as well as an expected significant increase in copper ($P < 0.001$) and fibrinogen ($P = 0.003$). In addition, there was a significant decrease in the plasma concentrations of lycopene ($P = 0.03$), α-carotene ($P = 0.02$), β-carotene ($P = 0.02$), and total carotenoids ($P = 0.01$). This decrease in circulating antioxidants may further compromise antioxidant status and may increase oxidative stress and damage in the elderly with inflammatory conditions.

In nine adult women (16), the consumption of 25 g of tomato puree (containing 7 mg of lycopene and 0.3 mg of β-carotene) for 14 consecutive days increased plasma and lymphocyte carotenoid concentration, and this was related to an improvement in lymphocyte resistance to an oxidative stress (500 μ*M*/l hydrogen peroxide for 5 min). Before and after the period of tomato intake, carotenoid concentrations were analyzed by HPLC and lymphocyte resistance to oxidative stress by the Comet assay, which detects DNA strand breaks. Intake of tomato puree increased plasma ($P < 0.001$) and lymphocyte ($P < 0.005$) lycopene concentration and reduced lymphocyte DNA damage by approximately 50% ($P < 0.0001$). β-Carotene concentration increased in plasma ($P < 0.05$) but not in lymphocytes after tomato puree consumption. An inverse relationship was found between plasma lycopene concentration ($r = -0.82$, $P < 0.0001$) and lymphocyte lycopene concentration ($r = -0.62$, $P < 0.01$) and the oxidative DNA damage. These data suggest that small amounts of tomato puree or other processed tomato products such as juices, soups, or pasta sauces added to the diet over a short period can increase carotenoid concentrations and the resistance of lymphocytes to oxidative stress.

Modulation of Cyclo-Oxygenase Pathways and Xenobiotic Metabolism

In one recent study (17), the normal diets of human volunteers were supplemented with either 15 mg/day β-carotene ($n = 25$), lycopene ($n = 23$), or lutein ($n = 21$) for 26 days in three independent double-blind, placebo-controlled supplementation studies. Supplementation with β-carotene increased plasma linoleic acid, but left the polyunsaturated:saturated (P:S) fatty acid ratio unaltered. In contrast, supplementation with lycopene reduced linoleic acid, which resulted in a large decrease in the P:S ratio. Lutein supplementation had no effect. It was concluded that neither β-carotene, lycopene, nor lutein supplementation engender antioxidant effects that lead to the widespread general conservation of plasma polyunsaturated fatty acids (PUFAs). β-Carotene and lycopene supplementation appear to interact with the metabolism of linoleic acid, the "essen-

tial" fatty acid, resulting in either an increase (β-carotene) or decrease (lycopene) in its plasma concentration. Alterations in plasma 18:2 or P:S ratios could ultimately lead to changes in tissue cellular membrane composition and hence to alterations in eicosanoid biosynthesis. More research is needed on the effects of lycopene and other carotenoids on xenobiotic metabolism (18) and on its effects on inducible cyclo-oxygenase activity.

Conclusion

There is clear evidence from epidemiologic studies that the intake of 400–600 g per day of fruits and vegetables is associated with a reduced risk of common aerodigestive cancers (19). Recent epidemiological studies indicate that increased serum α-carotene and lycopene levels are associated with a reduced risk of lung cancer, even among smokers (20). This observation is consistent with the lack of effect of smoking on serum lycopene levels while markedly decreasing β-carotene levels. This suggests a special protective role in lung cancer just as the localization of lycopene to the prostate gland suggests a special protective role there. Fruit and vegetable interventions at the level of 500 g per day are practical and result in significant changes in the levels of biochemical markers such as folic acid and homocysteine (21). The emerging science suggests that it is now time to make dietary changes as the scientific evidence continues to build (22).

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