**Cancer-preventive activities of tocopherols and tocotrienols**

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The cancer-preventive activity of vitamin E has been studied. Whereas some epidemiological studies have suggested a protective effect of vitamin E against cancer formation, many large-scale intervention studies with α-tocopherol (usually large doses) have not demonstrated a cancer-preventive effect. Studies on α-tocopherol in animal models also have not demonstrated robust cancer prevention effects. One possible explanation for the lack of demonstrable cancer-preventive effects is that high doses of α-tocopherol decrease the blood and tissue levels of δ-tocopherols. It has been suggested that γ-tocopherol, due to its strong anti-inflammatory and other activities, may be more effective in the prevention of cancer. Our recent results have demonstrated that a γ-tocopherol-rich mixture of tocopherols inhibits colon, prostate, mammary and lung tumorigenesis in animal models, suggesting that this mixture may have a high potential for applications in the prevention of human cancer. In this review, we discuss biochemical properties of tocopherols, results of possible cancer-preventive effects in humans and animal models and possible mechanisms involved in the inhibition of carcinogenesis. Based on this information, we propose that a γ-tocopherol-rich mixture of tocopherols is a very promising cancer-preventive agent and warrants extensive future research.

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**Introduction**

Vitamin E consists of a group of eight structurally related compounds: α-, β-, γ- and δ-tocopherols (α-, β-, γ- and δ-T) and α-, β-, γ- and δ-tocotrienols (α-, β-, γ- and δ-TT). All four tocopherols consist of a chromanol ring and a 16-carbon side chain, but they differ in the chromanol ring, whereas γ-T is dimethylated (at the 7- and 8-positions of the chromanol ring), whereas γ-T is tri-methylated (at the 5-, 7- and 8-positions of the chromanol ring), whereas γ-T is di-methylated (at the 7- and 8-positions at the 5-, 7- and 8-positions of the chromanol ring). Tocotrienols have the same substitution pattern of methyl groups on the chromanol ring (for α-, β-, γ- and δ-form) as tocopherols, but they have an unsaturated 16-carbon side chain with double bonds at the 3′-, 7′-, and 11′-positions (1,2).

Tocopherols cannot be synthesized in humans and animals, therefore, they need to be obtained from dietary sources. γ-T and α-T are the major dietary tocopherols present in the human diet. γ-T is the most consumed tocopherol, estimated to be consumed several times more than α-T (3). Tocopherols are plentiful in vegetable oils, such as oils from soybean, corn, sesame and cottonseeds, as well as nuts (4,5). Tocotrienols are present in trace amounts in oils derived from rice bran, barley, wheat germ and rye and are not consumed in large quantities in North America. Tocotrienols, however, are plentiful in palm oil (up to 800 mg/kg), mainly consisting of γ-T and α-T, and are consumed mostly in East-South Asia (6).

γ-T has been traditionally recognized as ‘the’ vitamin E because of its superior activity in the classical fertility-restoration assay and its higher blood levels over other tocopherols and tocotrienols. For these reasons, most of the studies on vitamin E have focused on γ-T; the distinct biological activities of other vitamin E molecules have not been studied to the same extent. Lately, it has been recognized that other tocopherols, such as γ-T and δ-T, as well as tocotrienols, have novel biological activities (7–9). As discussed in several reviews (7–9), γ-T has stronger anti-nitrative and anti-inflammatory activities than α-T and may be more effective in the prevention of cancer, as well as cardiovascular and neurodegenerative diseases. As will be discussed later, our recent results in animal models on the inhibition of colon, prostate and mammary carcinogenesis by a γ-T-rich mixture of tocopherols (γ-TmT, containing 59.3% γ-T, 25.4% δ-T, 13.5% α-T and 1.6% β-T) are very exciting (10–14). This review discusses our current understanding of the cancer-preventive and other activities of tocopherols and tocotrienols.

**Absorption and metabolism of tocopherols**

Dietary tocopherols are absorbed from the intestinal mucosa as the free phenolic form since esters are hydrolyzed by the pancreatic esterases prior to absorption. Tocopherols are incorporated into the chylomicrons and transported to the liver via the lymphatic system. Diet fat promotes transfer of vitamin E into the lymphatic system. The uptake of tocopherols into the liver is probably non-specific, but the transfer of tocopherols in the liver to very low-density lipoproteins is mediated by a specific α-T transfer protein (15,16). δ-T transfer protein in the liver selectively transfers α-T to very low-density lipoproteins; α-T is, therefore, preferentially secreted into the circulation and transferred to non-hepatic tissues (2). Due to their low affinity for α-T transfer protein, hepatic γ-T and δ-T are less efficiently transferred to very low-density lipoproteins. Therefore, smaller portions of γ-T and δ-T are found in the blood and tissues, and most of them are excreted in the feces.

The major route of tocopherol metabolism is through side-chain degradation, initiated with hydroxylation of the α-methyl group by cytochromes P450 4F or 3A and followed by five cycles of β-oxidation to cut off two-carbon units from the main chain in each cycle (1,17). A larger percentage of γ-T and δ-T than α-T is degraded through this pathway (18). The short side-chain metabolites, γ- and δ-carboxylethyl hydroxychroman (CEHC) (19,20), as well as lower levels of γ-carboxymethylbutyl hydroxychroman (21) were excreted in the urine in conjugated forms as glucuronides and sulfates. Metabolites of side-chain degradation of different chain lengths have been observed upon incubation of tocopherols and tocotrienols with HepG2 liver cancer cells (22). These metabolites have recently been characterized in mouse and human fetal and urine samples (23). These urinary metabolites may reflect dietary exposure of γ- and δ-T, vitamin E nutritional status and disease states, such as inflammation, or smoking that could affect tocopherol metabolism.

**Abbreviations:** α-, β-, γ- and δ-T; α-, β-, γ- and δ-tocopherol; α-, β-, γ- and δ-tocotrienol; AOM, azoxymethane; CEHC, carboxylethyl hydroxychroman; CI, confidence interval; DSS, dextran sulfate sodium; γ-TmT, γ-T-rich mixture of tocopherols; PPAR-γ, peroxisome proliferator-activated receptor-γ; RR, relative risk; α-TP, α-tocopheryl phosphate.
Anti-oxidative activities: trapping of reactive oxygen and nitrogen species

Vitamin E serves as antioxidants by preventing propagation of free radical reactions (2). Some in vitro studies have shown the superiority of α-T as an antioxidant over other tocopherols. Others, however, have found that antioxidant activities of γ-T are similar to or even greater than those of α-T (23). In addition to this direct antioxidant activity, tocopherols and their metabolites may serve as indirect antioxidants by activating NF-E2-related factor-2-related antioxidant enzymes. γ-Tocopherol quinone, the terminal oxidation product of γ-T, has been shown to be more effective than α-tocopherol quinone at increasing the transcription of activating transcription factor 4, a co-activator of oxidative stress during colon tumorigenesis was inhibited by γ-TmT in more polar solvents. Studied on tocopherols and human cancers

We have reviewed >70 publications on case–control, cohort and intervention studies examining the relationships between tocopherols and cancer risk at the four most common organ sites. The results are summarized in Table I and detailed information is provided in Supplementary Tables 1–4 (available at Carcinogenesis Online). In this section, we will first describe observational epidemiological studies on these four types of cancers and then the intervention studies, as they usually examined cancer risk at multiple organ sites.

Case–control and cohort studies

Colorectal cancer. Since 1992, there have been two case–control studies (39,40) and six cohort studies (41–46) on the relationship between dietary intake or blood levels of tocopherols and risk of colorectal cancer (Table I, Supplementary Table 1 is available at Carcinogenesis Online). Of the two case–control studies reported, one found an inverse association between supplementary vitamin E intake and colorectal cancer risk (39), but the other did not find a protective effect of dietary or supplementary vitamin E against colorectal cancer (40). This study, however, found a significant inverse association between the plasma α-T:γ-T ratio and large adenoma (≥1 cm) occurrence; the odds ratio for the highest versus lowest quintile was 0.36 with a 95% confidence interval (CI) of 0.14–0.95 (P = 0.02) (40). The authors suggested that the plasma α-T:γ-T ratio is a more sensitive indicator of tocopherol intake and a better predictor for cancer risk than plasma α-T levels, but the molecular basis is unclear. Nevertheless, an early meta-analysis of five prospective, nested case–control studies including 289 cases of colorectal cancer and 1267 matched controls showed that high plasma levels of α-T were associated with a modest decrease in the incidence of colorectal cancer (odds ratio: 0.6; 95% CI: 0.4–1.0) (44).

Of the six cohort studies, two studies showed an inverse association between vitamin E intake and colorectal cancer risk (45,46). For example, the Iowa Women’s Health Study (45) showed that a high intake of vitamin E was associated with a low risk of colon cancer (P for trend < 0.0001). This study also found that the protective effect was stronger in subjects under the age of 65 years than in subjects over the age of 65 (relative risk (RR): 0.37 for those 60–64 years old and 0.93 for those 65–69 years old).

Lung cancer. There have been four case–control studies (47–50) and three cohort studies (51–53) on the relationship between dietary or blood levels of tocopherols and risk of lung cancer since 1986 (Table I, Supplementary Table 2 is available at Carcinogenesis Online). Of the four case–control studies, three studies found lower serum α-T levels in lung cancer patients than those in matched controls (48–50). Two of these three studies found no difference in serum γ-T levels between lung cancer patients and the control subjects (48,49). Of the
three cohort studies, two studies found a significant inverse association between dietary intake of vitamin E and risk of lung cancer (51,52). In both of these studies, the protective effects were found in current smokers, suggesting a preventive effect of dietary vitamin E against insult from cigarette smoking.

Prostate cancer. There have been 14 case–control studies (49,54–66) and 9 cohort studies (53,67–74) on the relationship between dietary or blood levels of tocopherols and risk of prostate cancer since 1986 (Table I, Supplementary Table 3 is available at Carcinogenesis Online). Of the 14 case–control studies, seven showed an inverse association between dietary or blood levels of tocopherols and risk of prostate cancer (49,55,56,58,59,61,65). In two nested case–control studies (CLUE I and CLUE II), serum levels of γ-T, but not α-T, were significantly inversely associated with prostate cancer risk (56,75). In CLUE I, serum levels of γ-T were significantly lower in subjects who developed prostate cancers than control subjects (P = 0.02), but no dose–response trend was observed. A strong inverse association between γ-T and prostate cancer risk was observed in CLUE II (P = 0.0001) (56). Out of the nine cohort studies, six studies examined the association between dietary or supplementary vitamin E intake and prostate cancer risk, and all the studies did not find any significant association. In the National Institutes of Health-American Association of Retired Persons Diet and Health Study, dietary γ-T and δ-T were found to be significantly related to a reduced risk of advanced prostate cancer (RR: 0.68; 95% CI: 0.56–0.84 for γ-T and RR: 0.8; 95% CI: 0.67–0.96 for δ-T), but supplemental vitamin E (α-T) intake beyond dietary sources was not related to prostate cancer risk (67).

Breast cancer. There have been 15 case–control studies since 1992 (Table I, Supplementary Table 4 is available at Carcinogenesis Online). Of the eight case–control studies examining an association between vitamin E intake and breast cancer risk (76–83), six studies found a significant inverse association (76–80,82). Out of the seven case–control studies examining an association between serum α-T and γ-T levels and breast cancer risk, only one study found a significant inverse association with both α-T and γ-T levels (84). The other six studies did not show such an association (85–90). All the nine reported cohort studies found no association between vitamin E intake and breast cancer risk (91–99).

Collectively, the results from human case–control and cohort studies are inconsistent. Some studies showed a clear inverse association between tocopherol intake and cancer risk, whereas others showed no such association.

Intervention studies
The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was initially designed to investigate the prevention of lung cancer in male smokers with a daily supplement of 50 mg of all-racemic-α-tocopheryl acetate and 20 mg of β-carotene in a two-by-two design (100). The α-T supplementation for 5–8 years did not produce a significant effect on the incidence of lung cancer (100). It lowered the incidence of colorectal cancer, but the result was not statistically significant (101). Additional studies found no significant association between colorectal cancer risk and dietary vitamin E, dietary α-T, dietary γ-T or serum α-T levels (42). During the 6-year post-trial period, no post-intervention effect of the supplement on colon cancer risk was found (102).

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention study showed that α-T supplementation (50 mg daily for 5–8 years) was significantly associated with the reduced incidence of prostate cancer and that higher serum α-T was associated with a reduced risk of prostate cancer (RR: 0.80; 95% CI: 0.66–0.96 for highest versus lowest quintile; P trend = 0.03) (68,102,103). These results encouraged the launching of the Selenium and Vitamin E Cancer Prevention Trial, a clinical trial to determine if one or both of these substances can help prevent prostate cancer when taken as dietary supplements. The recently published results indicated that selenium (200 μg/d from L-selenomethionine) and vitamin E (400 IU/d of all-rac-α-tocopheryl acetate), taken alone or together for an average of 5 years, did not prevent prostate cancer (104). However, the α-T supplementation caused a 50% decrease in median plasma γ-T levels (104).

In the Women’s Health Study with 39 876 healthy US women aged 45 years or older, the administration of 600 IU of natural-source vitamin E (α-T) on alternate days did not significantly affect the incidence of colon, lung or total cancers (105). In the recently published results from the Physicians’ Health Study II Randomized Control Trial, supplementation with vitamin E (400 IU synthetic α-T every other day) or vitamin C (500 mg synthetic ascorbic acid) to physicians for 8 years did not reduce the risk of prostate cancer or all other cancers (106). The results of these large, long-term trials with high doses of α-T are disappointing. There are at least two interpretations of the results: (i) supplementation of a nutrient to a population that is already adequate in this nutrient may not produce any beneficial effects and (ii) supplementation of a large quantity of α-T decreases the blood and tissue levels of γ-T and δ-T, which have been suggested to have unique cancer-preventive activities (7–9,23,107,108). Based on our results from animal models, we believe that a mixture of tocopherols may produce more beneficial effects than individual tocopherols.

Inhibition of tumorigenesis in animal models by tocopherols
Most of the animal studies that have been conducted used α-T and its synthetic analogs. Table II summarizes the results of 32 studies published since 1980. More detailed information is provided in Supplementary Tables 5–8 (available at Carcinogenesis Online). The following is a summary of studies on four common organ sites of carcinogenesis.

Colon tumorigenesis
There have been a total of 12 studies on the effect of tocopherols on colon tumorigenesis and aberrant crypt foci formation (Table II, Supplementary Table 5 is available at Carcinogenesis Online). Ten studies were on α-T and its synthetic analogs; only one showed

| Table I. Number of studies on the risk of human cancers and the dietary intake or blood levels of total tocopherols |
|-------------|-------------|-------------|-------------|
| Case–control studies | Cohort studies | Intervention studies |
| Risk reduction | No association | Risk reduction | No association | Risk reduction | No association |
| Colon | 1 | 1 | 2 | 4 | 0 | 4 |
| Lung | 3 | 1 | 2 | 1 | 0 | 4 |
| Prostate | 7 | 7 | 3 | 6 | 1 | 3 |
| Breast | 7 | 8 | 0 | 9 | 0 | 0 |

Results based on a review of studies published since 1986.

| Table II. Number of animal studies showing protective or no protective effects of tocopherols on tumor formation in different organs |
|-------------|-------------|-------------|-------------|
| Site | α-Tocopherol or its analogs | Other tocopherols |
| | Protective effect | No protective effect | Protective effect | No protective effect |
| Colon | 1 | 9 | 2* | 0 |
| Lung | (1) | 0 | 0 | 0 |
| Prostate | 2 (1) | 1 (2) | 1* | 0 |
| Mammary gland | 4 (5) | 1 (0) | 2* | 0 |

Results based on a review of studies published since 1980. The number of xenograft studies is in parentheses.

aStudy with γ-tocopherol-enriched mixed tocopherols.

bStudy for the effect on metastasis to the lung.
Prostate tumorigenesis and transplanted prostate cancer cells

Of a total of six studies on prostate tumorigenesis and transplanted prostate cancer cells (11), three studies were on the effect on prostate carcinogenesis in rats (119–121), and the other three studies were on their effects on the growth of human prostate cancer cells in nude mice (122–124); the results are inconsistent. One study found that treatment with α-tocopheryl succinate resulted in a significant reduction of prostate cancer incidence in a transgenic mouse model, but the diet used also contained other agents (800 IU of α-tocopheryl succinate, 200 μg of seleno-DL-methionine and 50 mg of lycopene). Our recent studies demonstrated that administration of 0.1% of γ-TmT in the diet of TRAMP mice significantly inhibited the development of palpable prostate tumors and prostate intraepithelial neoplasia. The treatment also upregulated NF-E2-related factor-2 and related detoxifying and anti-oxidative enzymes (12). As discussed previously, the induction of anti-oxidant enzymes may be due to the action of α-tocopheryl quinone (24).

Mammary tumorigenesis

Of five studies on mammary tumorigenesis, four studies showed a protective effect (113,125–127) but one study showed no effect (128) (Table II, Supplementary Table 7 is available at Carcinogenesis Online). Recently, we demonstrated that dietary administration of γ-TmT (0.1% in the AIN76A diet) significantly inhibited N-nitrosourea-induced mammary tumorigenesis in rats (13). We found that mammary tumor growth and tumor multiplicity, as well as a proliferation marker, proliferating cell nuclear antigen, were markedly decreased by administration of γ-TmT. In a subsequent study with γ-TmT, administration of 0.1, 0.3 or 0.5% γ-TmT dose dependently suppressed mammary tumor development and growth. Tumor multiplicity was also significantly reduced by all three different doses of γ-TmT. The inhibition of mammary tumorigenesis was associated with increased expression of p21, p27, cleaved caspase-3 and peroxisome proliferator-activated receptor (PPAR)-γ, whereas Akt and the estrogen-dependent signaling pathways in mammary tumors were significantly decreased by γ-TmT treatment (14).

Lung cancer

There is only one publication on tocopherol and lung cancer reporting that supplementation with α-T and mammary tumorigenesis in intravenously inoculated murine colon adenocarcinoma cells in BALB/C mice (129) (Table II, Supplementary Table 8 is available at Carcinogenesis Online). Using γ-TmT at 0.3% in the AIN93M diet, we recently observed growth inhibition of CL-13 murine lung cancer cells growing syngeneically in A/J mice (130). We also demonstrated that dietary γ-TmT (0.3%) inhibited growth of H1299 human lung cancer cells in xenografts in nude mice as well as inhibited lung tumorigenesis in A/J mice induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane plus benzo[a]pyrene (G.Lu, H.Xiao, G.Li, Y.K.Chen, J.J.Hao, S.Loy and C.S.Yang, submitted). The strongest inhibitory effect was observed with γ-TmT treatment starting at the beginning of the carcinogenesis experiment. Overall, results on the effect of α-T on animal carcinogenesis are inconsistent, with most studies showing no inhibition (e.g. in colon tumorigenesis) and some showing inhibition (e.g. in mammary tumorigenesis). On the other hand, recent studies with γ-TmT have consistently shown inhibitory effects against tumorigenesis in the colon, mammary gland, prostate and lung. Therefore, γ-TmT appears to be a promising agent for future investigation.

Possible cancer prevention mechanisms

Many studies have been conducted on the biological activities of tocopherols. The cancer prevention activity of tocopherols may be due to the following activities or a combination of these activities. The most commonly recognized are the anti-oxidative activities of tocopherols. The quenching of reactive nitrogen species by γ-T and δ-T, as well as the inhibitory activities of their metabolites against COX-2, make γ-T and δ-T stronger anti-inflammatory and anti-carcinogenic agents than α-T. γ-T and δ-T are also more effective at modulating the activities of certain receptors, signal transduction pathways and metabolic pathways that may contribute to the higher cancer-preventive activity of γ-T and δ-T. Some of the studies are described below.

Anti-oxidative activities and trapping of reactive nitrogen species

As was discussed in Anti-oxidative Activities: Trapping of Reactive Oxygen and Nitrogen Species, the anti-oxidative action is a common feature of all the forms of tocopherols, whereas γ-T and δ-T can effectively trap reactive nitrogen species. These activities have been demonstrated in our studies with γ-TmT in the AOM-induced/DSS-induced colon carcinogenesis model (11) and probably exist in other carcinogenesis systems.

Inhibition of COX-2 and anti-inflammatory activities

γ-T was shown to be more effective than α-T at inhibiting cyclooxygenase activity (131) and formation of pro-inflammatory eicosanoids (131–133). γ-T reduced prostaglandin E2 synthesis in both lipopolysaccharide-stimulated RAW264.7 macrophages and IL-1β-treated A549 human epithelial cells with an the concentration that causes 50% inhibition of 7.5 and 4 μM, respectively (131). The major metabolite of γ-T, γ-CEHC, also exhibited an inhibitory effect, with an the concentration that causes 50% inhibition of ~30 μM in these cells. However, α-T, at 50 μM, only slightly reduced prostaglandin E2 formation in macrophages but had no effect in epithelial cells. The inhibitory effects of γ-T and γ-CEHC were due to the inhibition of COX-2 activity, rather than the protein expression or substrate availability. The inhibitory potency of γ-T and γ-CEHC was diminished by an increase in arachidonic acid concentration, suggesting that they compete with arachidonic acid at the active site of COX-2. Recent studies showed that long-chain carboxychromanol metabolites of vitamin E inhibited COX-2 more potently than shorter side-chain metabolites, whereas the sulfated carboxychromanols were ineffective (134). The long-chain metabolites in conditioned medium from γ-T, and even more so from δ-T, were more effective than conditioned medium from α-T, possibly because α-T was metabolized to long-chain metabolites to a lesser extent (135,136).

Some studies suggest that mixtures of tocopherols are superior to a single tocopherol at inhibiting inflammation. In subjects with metabolic syndrome (n = 20 per group), supplementation with a combination of γ-T and α-T each at 800 mg/day for 6 weeks resulted in more pronounced decreases in C-reactive protein, tumor necrosis factor-α and nitrotyrosine levels than supplementation with γ-T or α-T (800 mg/day) individually (137). We recently demonstrated that administration of γ-TmT to AOM-treated/DSS-treated mice reduced the colon inflammation index to 52% of the control and decreased levels of prostaglandin E2 and LTB4 in the colon and plasma (11).

Modulation of nuclear receptors

PPAR-γ, which belongs to the nuclear receptor family, is known to be important for inhibition of cell proliferation and induction of apoptosis in breast cancer. Upregulation of PPAR-γ may be one of the mechanisms for anti-carcinogenic action. Two studies have shown that γ-T is more effective than α-T at modulating the expression of...
PPAR-\(\gamma\) (138,139). Campbell et al. (138) showed that treatment of SW 480 colon cancer cells with \(\alpha\)-T and \(\gamma\)-T (5–10 \(\mu\)M) increased the messenger RNA and protein levels of PPAR-\(\gamma\), with a more pronounced effect produced by treatment with \(\gamma\)-T. De Pascale et al. (139) showed that all four natural tocopherols, \(\alpha\)-T, \(\beta\)-T, \(\gamma\)-T and \(\delta\)-T, increased transcriptional activity of PPAR-\(\gamma\) in NCTC 2544 human keratinocytes cell line, and \(\gamma\)-T displayed the strongest activity. Treatment with \(\alpha\)-T, \(\beta\)-T, \(\gamma\)-T and \(\delta\)-T also increased protein levels of PPAR-\(\gamma\) and transglutaminase-1, a downstream protein of PPAR-\(\gamma\) involved in terminal keratinocytes differentiation. Recently, we found that \(\gamma\)-TmT, \(\gamma\)-T and \(\delta\)-T activated PPAR-\(\gamma\) transcription in estrogen receptor-positive breast cancer cell lines, MCF-7 and T47D cells; \(\delta\)-T was more active than \(\gamma\)-T, whereas \(\alpha\)-T was not active (14).

Pregnane X receptor is a nuclear receptor that recognizes xeno-biotics, and it mediates the induction of genes involved in oxidation, conjugation and transportation of xenobiotics. In HepG2 cells, the transfected human pregnane X receptor was most strongly activated by \(\alpha\)-T and \(\gamma\)-TT followed by \(\delta\)-T, \(\alpha\)-T and \(\gamma\)-T. These results suggest a potential effect of individual forms of vitamin E on the metabolism of certain drugs and environmental chemicals (140). Prolonged treatment with \(\alpha\)-T may induce CYP3A and enhance the side-chain degradation of tocopherols; this may lead to the lowering of blood levels of \(\gamma\)-T (2, 9, 104).

**Mechanisms for inhibition of cell growth and induction of apoptosis in cell culture**

\(\gamma\)-T has been shown to be more effective than \(\alpha\)-T at inhibiting growth of colon, breast, prostate and lung cancer cells in culture (141–145). \(\gamma\)-T decreased the number of cells in S phase more effectively than \(\alpha\)-T in human colon and prostate cells in culture by decreasing protein levels of cyclin D1 and cyclin E (key regulators of the G1–S transition) as well as p27kip1, p21cip1 and p16ink4a (142). Treatment of human glioma cells with \(\gamma\)-T and \(\alpha\)-T inhibited cell growth, partially by increasing protein levels of integrin \(\alpha\)5 and \(\beta\)1 (146). Overexpression of integrin \(\alpha\)5 and \(\beta\)1 has been reported to inhibit cell cycle progression.

\(\gamma\)-T has also been shown to be effective at inducing apoptosis in cancer cells (141,144). \(\gamma\)-T (10–50 \(\mu\)M) or its combination with \(\delta\)-T induced apoptosis in androgen-sensitive prostate LNCaP (but not in androgen-resistant PC-3 cells) by the induction of cytochrome c release, activation of caspase-9 and caspase-3, cleavage of poly-ADP-ribose polymerase and involvement of caspase-independent pathways (143). \(\gamma\)-T treatment also caused significant accumulation of dihydrolipamide and dihydrosphingosine, and specific inhibitors of key enzymes of de novo synthesis of sphingolipids significantly protected cells from \(\gamma\)-T-induced apoptotic pathway (144). The study suggests that \(\gamma\)-T induced apoptosis by interrupting the de novo sphingolipid pathway in a prostate cancer cell line. Lyons et al. (147), however, reported that \(\gamma\)-T (60 \(\mu\)M), but not \(\alpha\)-T, inhibited sterol-induced apoptosis in human monocyte U937 cells.

Our study with \(\gamma\)-TmT, as well as individual isoforms of tocopherols (10–100 \(\mu\)M concentration), demonstrated a dose-dependent inhibition of the estrogen-induced cell proliferation of the estrogen receptor-positive breast cancer cell line, MCF-7. \(\alpha\)-T did not significantly inhibit the growth of estrogen receptor-positive human breast cancer cell line, MCF-7, whereas \(\gamma\)-T, and more strikingly \(\delta\)-T, inhibited estrogen-induced cell proliferation in a dose-dependent manner (14).

**Other possible mechanisms of action**

\(\alpha\)-T has been shown to exert anti-proliferative activity independent of its traditional antioxidant activity. \(\alpha\)-T activated protein phosphatase 2A resulting in dephosphorylation and decreased protein kinase C activity (148,149). Additionally, \(\alpha\)-T inhibited expression of the CD36 scavenger receptor which is a receptor involved in uptake of oxidized low-density lipoprotein and atherosclerosis progression (150). Short-term dietary supplementation with high doses of vitamin E was shown to increase \(\gamma\) helper 1 cytokine production in patients with advanced colorectal cancer (151). In this study, supplementation of vitamin E (750 mg/day) for 2 weeks resulted in increased CD4:CD8 ratios and enhanced capacity of T cells for producing the T helper 1 cytokines interleukin 2 and interferon-\(\gamma\).

\(\alpha\)-Tocopheryl phosphate (\(\alpha\)-TP) has recently been studied because of its potentially stronger anti-proliferative activity than that of \(\alpha\)-T and its presence in food and animal tissues (152). \(\alpha\)-TP was more effective than \(\alpha\)-T at inhibiting cell proliferation (153). A mixture of \(\alpha\)-TP and di-\(\alpha\)-TP suppressed cell proliferation and CD36 levels in aortic smooth muscle and monocytic leukemia cells at a concentration lower than the effective concentration of \(\alpha\)-T. It was reported that \(\alpha\)-TP-induced apoptosis in the osteosarcoma cell line MG-63 (154), whereas it was demonstrated to have cardioprotective and anti-apoptotic activity through the Akt survival pathway in a rat model of myocardial infarction (155). Although the physiological functions of the phosphorylated forms of tocopherol still remain to be established, the cancer-preventive activities of \(\gamma\)-TP and \(\delta\)-TP are worth investigating. Since tocopherols are known to be embedded in lipid bilayers of cell membranes, it is interesting to consider that the phosphorylated form (tocopherol phosphate) may be able to move to the cytosol and possibly the nucleus to trigger different biochemical reactions.

**Studies on tocotrienols and cancer in humans, animals and cells**

Tocotrienols, the vitamin E isomers with unsaturated side chains, have been shown to display stronger anticancer activities in vitro than tocopherols with \(\gamma\)- and \(\delta\)-TT exhibiting more anticancer activities than \(\alpha\)-TT (156–160). This subject has been reviewed recently (161). Although TTs possess antioxidant activity (162–164), the anticancer activity of TTs may be independent from its antioxidant activity because some redox-silent TT derivatives still exhibit antitumorogenic properties (165,166). For example, treatment of human lung adenocarcinoma cells with a redox-silent analog of \(\alpha\)-TT led to accumulation of cells in the G1 phase of the cell cycle followed by apoptosis (165). This same redox-silent analog inhibited chemoresistant mesothelioma cell growth (167).

Recent results suggest that TTs affect many signaling pathways in cancer cells, including NF-kB-mediated pathways, phosphatidylinositol-2 kinase/phosphoinositide-dependent/Akt, Raf/Erk and c-Jun N-terminal kinase-related pathways (168–172). TTs also mediate many, cellular processes including the reduction of DNA damage (173), activation of apoptosis (174), induction of cell cycle arrest (175), stabilization of the proteasome (176), and downregulation of telomerase activity (177). TT-induced apoptosis was observed in many different cancer cell lines (178–181), and usually involved proteins related to mitochondrial stress, such as alteration of Bcl-family proteins and caspases (182,183). However, the caspase activation induced by TTs may also involve mechanisms independent of death receptor and mitochondrial stress (174,184). In addition to apoptosis, \(\gamma\)-T and \(\delta\)-TTs also induced autophagy through a mitochondrial permeability transition pore opening-dependent, but caspase-independent, mechanism, suggesting the involvement of autophagy in TT-mediated cell death (185).

Other important anticancer properties of TTs are their anti-angiogenic activity and their ability to inhibit cancer invasion and metastasis. The anti-angiogenic effect of \(\delta\)-TT is attributable to the regulation of phosphatidylinositol-2 kinase/phosphoinositide-dependent kinase/Akt signaling and hypoxia-induced VEGF secretion as well as to the induction of a stress response in endothelial cells, partly associated with reactive oxygen species generated by \(\delta\)-TT (186,187). \(\gamma\)-TT inhibited cancer cell invasion through downregulation of matrix metalloproteinase-2 and -9 and upregulation of tissue inhibitor of metalloproteinase-1 and -2 (188). \(\gamma\)-TT treatment also led to the suppression of mesenchymal markers and the restoration of epithelial markers, which are associated with inhibition of cell invasion (189). The inhibition of tumor formation and growth has been studied in several mouse and rat models. In carcinogenesis models, oral administration of a 0.08% TT mixture in drinking water significantly

Cancer-preventive activities of tocopherols and tocotrienols
suppressed spontaneous liver carcinogenesis in male C3H/He mice and glycerol-induced lung tumor promotion in 4NQO-initiated ddY mice (175). Other studies demonstrated that TTs inhibited the severity of cell damage in hepatocarcinogenesis (190,191). However, it was also reported that TTs did not have a significant effect on chemically induced rat mammary tumor latency and multiplicity (128). In a xenograft tumor model with B16 melanoma cells, γ-γTT suppressed tumor growth and extended survival time of the host C57BL mice (159). Dietary γ-TT and δ- TT significantly delayed tumor growth in C3H/HeN mice implanted with murine hepatoma MH134 cells (192). The anticancer effect of TTs in animal studies requires further exploration.

Concluding remarks

The association of low vitamin E status with increased cancer risk as described above and observed in other human epidemiological studies (193–197) suggests the importance of these anti-oxidative nutrients in modulating cancer incidence. However, the results of most of the animal and human studies with α-T supplementation, as reviewed above, have not yielded supportive evidence. It is possible that tocopherols may reduce cancer risk when supplemented in populations with low vitamin E status. When given to humans and animals with adequate vitamin E nutrition, the cancer-preventive effects of tocopherols could be due to actions other than the anti-oxidative activity of α-T. In this aspect, as reviewed above, the most abundant γ-T is superior to α-T in the trapping of reactive nitrogen species, inhibition of COX-2 activity, activation of PPAR-γ and suppression of inflammation, δ-T, which is more abundant than α-T in some oils, also has some of these activities. We propose that a mixture of tocopherols, at ratios similar to those in our diet, could be a better cancer chemopreventive agent. This idea is supported by our recent results demonstrating that γ-TmT inhibited colon, mammary, prostate and lung carcinogenesis in rodent models as well as inhibiting growth of lung and prostate cancer xenograft tumors (10–14).

It has been suggested that γ-T is the major cancer-preventive form of vitamin E (8,107,198). However, the cancer-preventive activity of pure γ-T or δ-T still remains to be demonstrated. It is known that high levels of α-T intake can decrease the blood and tissue levels of γ-T. Whether high levels of dietary γ-T or δ-T can also decrease the blood and tissue levels of α-T remains to be investigated. It would be interesting to determine the contributions of each of the major forms of tocopherols (α-, γ- and δ-) to cancer prevention and the possible interactions among these tocopherols as well as the mechanisms involved. In practical application, γ-TmT is probably the most promising agent to use. γ-TmT, a by-product in the refining of soybean oil, contains γ-T, α-T, δ-T and β-T in ratios approximate to those in dietary vegetable oils. Because it is readily available and inexpensive, γ-TmT and similar tocopherol preparations have a high potential for practical application and deserve further investigation in animal models and human trials.

In future epidemiological studies, more attention should be paid to dietary intake, blood and tissue levels of all major forms of tocopherols, as well as their ratios. Since γ-T and δ-T are more readily side-chain degraded, urinary levels of γ- and δ-CEHC may be explored as possible markers for the consumption of γ-T and δ-T and physiological conditions that affect their metabolism. Well-designed human intervention trials with γ-TmT may yield more definitive information on the cancer-preventive activities of tocopherols.

Supplementary material

Supplementary Tables 1–8 can be found at http://carcin.oxfordjournals.org/

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References


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