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Cancer Prevention by Different Forms of Tocopherols

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ABSTRACT

Low vitamin E nutritional status has been suggested to increase cancer risk. However, recent large-scale human trials with high doses of alpha-tocopherol (α -T) have produced disappointing results. This points out the need for a better understanding of the biological activities of different forms of tocopherols. Using a tocopherol mixture that is rich in γ -T (γ -TmT), we demonstrated the inhibition of colon carcinogenesis in mice, and the inhibition is associated with decreased levels of 8-isoprostane, nitrotyrosine, prostaglandin E2 and leukotriene B4. Dietary 0.3% γ -TmT also inhibited chemically induced lung tumorigenesis in the A/J mice as well as the growth of lung cancer cells in xenograft or allograft tumors; the inhibition was associated with a reduction of oxidative/nitrosative stress. δ -T was found to be more active than γ -T in the inhibition of cancer cell growth in culture and lung cancer xenograft tumors as well as in azoxymethane-induced colon aberrant crypt foci formation in rats, whereas α -T was ineffective. Analysis of the levels of tocopherols and their metabolites in blood and tissues suggests that metabolites of δ -T and γ -T contribute to their inhibitory activity. These studies demonstrate the broad cancer preventive activity of γ -TmT and higher activity of δ -T.

Key words: tocopherols, vitamin E, inhibition, colon cancer, lung cancer

INTRODUCTION

Tocopherols, collectively known as vitamin E, are a family of fat-soluble phenolic compounds. Each tocopherol contains a chromanol ring system and a phytol chain containing 16 carbons. Depending upon the number and position of methyl groups on the chromanol ring, they exist as α -, β -, γ -, or δ -tocopherols (α -, β -, γ -, or δ -T)⁽¹⁾. The structures of these tocopherols are shown in Figure 1. α -T is trimethylated at the 5-, 7- and 8-positions of the chromanol ring, whereas γ -T is dimethylated at the 7- and 8-positions and δ -T is methylated at the 8-position. The unmethylated carbons at 5- and 7-positions are electrophilic centers, which effectively trap reactive oxygen and nitrogen species (RONS). The formation of 5-nitro- γ -T, 5-nitro- δ -T, 7-nitro- δ -T, and 5,7-dinitro- δ -T have been reported⁽²⁾. The hydrocarbon tail and ring structure provide the lipophilicity for tocopherols to be incorporated into the lipid bilayers of biological membranes. The phenolic group in the chromanol moiety effectively quenches lipid free radicals by one electron reduction. The resulting tocopherol phenoxy radical, can be reduced by ascorbic acid or glutathione to regenerate the tocopherol molecule. This is probably the most important physiological antioxidant mechanism to protect the integrity of biological membranes. In this article, we will briefly discuss the cancer preventive activities of different forms of tocopherols based on results from human studies and our recent results from studies in animal models.

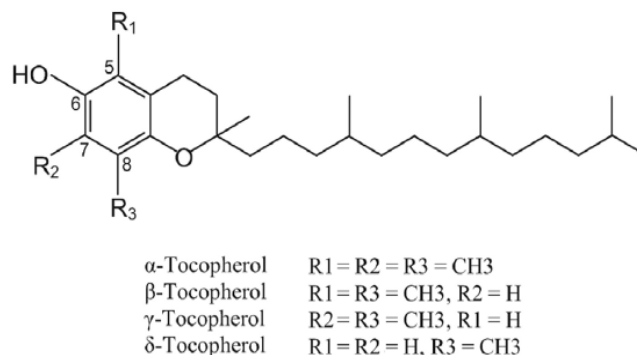


Figure 1. Structures of tocopherols.

HUMAN STUDIES ON TOCOPHEROLS AND CANCER

Because of the involvement of RONS in carcinogenesis, tocopherols, as effective antioxidants, are expected to protect against carcinogenesis. There is evidence to support this concept. For example, of the three reported cohort studies on lung cancer, two studies found a significant inverse association between dietary intake of vitamin E and risk of lung cancer⁽³⁾. In both of these studies, the protective effects were found in current smokers, suggesting a protective effect of vitamin E against insult from cigarette smoking. In four case-control studies on lung cancer, three studies found lower serum

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α -T levels in lung cancer patients than in matched controls⁽³⁾. In a recent case-control study, Mahabir *et al.*⁽⁴⁾ observed that the odds ratios of lung cancer for increasing quartiles of dietary α -T intake were 1.0, 0.63, 0.58, and 0.39, respectively (p for trend < 0.0001)⁽⁴⁾. The authors concluded that α -T accounts for 34 - 53% reduction in lung cancer risk. Since the intake of γ -T was also increased in proportion to α -T, and at higher quantities, the beneficial effect could also be due to γ -T or the combined effects of all the forms of tocopherols. γ -T is 3 to 4 times more abundant than α -T and δ -T could also be more abundant than α -T in our diet. γ -T and δ -T have also been shown to be more effective in trapping reactive nitrogen species⁽²⁾.

Because α -T is the most abundant form of tocopherols in blood and tissues and has the highest activity in the classical fertility-restoration assay, α -T is generally considered to be "the vitamin E"⁽¹⁾. Therefore, many studies on vitamin E have been conducted with α -tocopheryl acetate. The results from several large-scale intervention studies with α -T, however, have been disappointing⁽⁵⁻⁸⁾. For example, in the recent Selenium and Vitamin E Cancer Prevention Trial (SELECT), taking 400 IU all-rac α -tocopheryl acetate or 200 μ g selenium from L-selenomethionine, or both, daily for an average of 5 years, did not prevent prostate or other cancer⁽⁷⁾. In the recently published results on the follow-up (for 7-12 years) of this study, subjects receiving α -tocopherol acetate had a hazard ratio of 1.17 for developing prostate cancer⁽⁸⁾. It was noted that the α -T supplement caused a 50% decrease in median plasma γ -T levels⁽⁷⁾. A possible interpretation of the result is that supplementation of a nutrient to a population that is already adequate in this nutrient may not produce any beneficial effects. It is also possible that supplementation of a large quantity of α -T decreases the blood and tissue levels of γ -T, which has been suggested to have stronger anti-inflammatory and cancer preventive activities^(3,9-13). The exact reasons for these negative results are not known. Nevertheless, the disappointing outcome of these large-scale trials reflects our lack of understanding of the biological activities of tocopherols and points to the need for systematic studies of the disease preventive activities of the different forms of tocopherols.

INHIBITION OF TUMORIGENESIS BY γ -TMT IN ANIMAL MODELS

Previous cancer prevention studies in different animal models with pure α -T have obtained inconsistent results⁽³⁾. On the other hand, recent studies from our research team at Rutgers University have demonstrated the inhibition of cancer formation and growth in the lung, colon, mammary gland, and prostate by a tocopherol mixture that is rich in γ -T (γ -TmT)⁽¹⁴⁻²¹⁾. γ -TmT is a by-product in the distillation of vegetable oil and usually

contains (per g) 130 mg α -T, 15 mg β -T, 568 mg γ -T, and 243 mg δ -T. Some studies on lung and colon cancer are discussed in detail in the following sections.

I. γ -TmT Inhibits Lung Carcinogenesis

In studying the lung cancer preventive activity of γ -TmT, we treated A/J mice (6 weeks old) with a tobacco carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), plus benzo[a]pyrene (B[a]P), a ubiquitous environmental pollutant, at doses of 2 μ mol each, by oral gavage weekly from Weeks 1 to 8. At Week 19, the mice in the control group (on AIN93M diet) developed 21 tumors per mouse⁽¹⁴⁾. Treatment of the mice with 0.3% γ -TmT in the diet during the entire experimental period lowered the tumor multiplicity to 14.8 (30% inhibition, p < 0.05). γ -TmT treatment also significantly reduced the average tumor volume and tumor burden by 50% and 55%, respectively⁽¹⁴⁾.

In a second study, lung tumorigenesis was induced by NNK (i.p. injection of 100 mg/kg on Week 1 and 75 mg/kg on Week 2). The 0.3% γ -TmT diet was given during the carcinogen-treatment stage, the post-initiation stage, or the entire experimental period. γ -TmT treatment during these three time periods all reduced the tumor multiplicity (17.1, 16.7 and 14.7 per mouse, respectively, as compared to 20.8 in the control group; p < 0.05). Moreover, the tumor burden was significantly reduced by γ -TmT treatment given during the tumor initiation stage or during the entire experimental period by 36% and 43% inhibition, respectively⁽¹⁴⁾.

In the NNK plus B[a]P-treated model, dietary γ -TmT treatment significantly increased the apoptotic index (based on cleaved-caspase-3 positive cells) from 0.09 to 0.25% in the lung tumors; whereas the treatment did not affect apoptosis in non-tumorous lung tissues. Dietary γ -TmT treatment also significantly decreased the percentage of cells with positive immunostaining for 8-hydroxydeoxyguanosine (8-oxo-dG) (from 26 to 17%), a marker for oxidative DNA damage, as well as for γ -H2AX (from 0.51 to 0.23%), a reflection of double-strand break-induced DNA repair. The plasma levels of prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) were markedly elevated in the tumor-bearing A/J mice at Week 19 as compared to mice that received no carcinogen treatment. γ -TmT treatment resulted in lower plasma levels of PGE2 (by 61%, p < 0.05) and LTB4 (by 12.7%, p < 0.1). These results suggest the antioxidant and anti-inflammatory activities of γ -TmT. The anti-angiogenic activity of dietary γ -TmT was demonstrated with anti-endothelial cell CD31 antibodies. CD31-labeled capillary clusters and blood vessels were observed mainly in the peripheral area of the lung adenomas, and dietary γ -TmT reduced the microvessel density (blood vessels/mm²) from 375 to 208 (p < 0.05)⁽¹⁴⁾.

II. δ - and γ -Tocopherols and γ -TmT Inhibit Xenograft Lung Tumorigenesis and Tumor Growth

When 0.3% γ -TmT was given to NCr nu/nu mice in the diet one day after implantation of human lung H1299 cells (1×10^6 cells injected s.c. per site to both flanks of the mouse), an inhibition of xenograft tumor growth was observed⁽¹⁴⁾. After 6 weeks, the tumor size and weight were significantly reduced by 56 and 47%, respectively, as compared to the control group. The γ -TmT treatment also caused a 3.3-fold increase in apoptotic index as well as a 52% decrease in 8-oxo-dG-positive cells and a 57% decrease in γ -H2AX-positive cells in the xenograft tumors. Strong cytoplasmic staining of nitrotyrosine was observed in xenograft tumors, and the staining intensity was decreased by 44% in mice that received γ -TmT. The γ -TmT treatment also reduced the plasma LTB4 level by 36.5% ($p < 0.05$)⁽¹⁴⁾.

In a similar experiment, the effectiveness of different forms of pure tocopherols in the inhibition of H1299 xenograft tumor growth was compared⁽¹⁵⁾. Pure δ -T was most effective, showing dose-response inhibition when given at 0.17 and 0.3% in the basal AIN-93M diet. γ -TmT and pure γ -T were less effective, but α -T was not effective at diet levels of 0.17 and 0.3%. Studies of H1299 cells in culture also showed that δ -T was more effective than γ -TmT and γ -T in inhibiting cell growth, whereas α -T was not effective⁽¹⁴⁾.

In another transplanted tumor study, dietary 0.1 and 0.3% γ -TmT were found to inhibit the growth of subcutaneous tumors (formed by injection of murine lung cancer CL13 cells) in A/J mice by 54 and 80%, respectively, on Day 50⁽¹⁶⁾.

III. Tocopherols Inhibit Colon Inflammation and Tumorigenesis

Previous studies concerning the effect of α -T on colon carcinogenesis have yielded mostly negative results⁽³⁾. Recently, we studied the effect of γ -TmT in the colons of mice that had been treated with azoxymethane (AOM) and dextran sulfate sodium (DSS)⁽¹⁷⁾. Dietary γ -TmT treatment (0.3% in the diet) resulted in a significantly lower colon inflammation index (52% of the control) on Day 7, and reduced the number of colon adenomas (to 9% of the control) on Week 7. γ -TmT treatment also resulted in higher apoptotic indexes in adenomas, lower PGE2, LTB4, and nitrotyrosine levels in the colon, and lower PGE2, LTB4, and 8-isoprostane levels in the plasma on Week 7. In a second experiment, with AOM/DSS-treated mice sacrificed on Week 21, dietary γ -TmT treatment significantly inhibited adenocarcinoma and adenoma formation in the colon (to 17 - 33% of the control). In a third experiment, mice received dietary treatment with 0, 0.1, and 0.3% γ -TmT in the AIN 93M basal diet. One week later, 1% DSS was given to mice in drinking water for one week to induce

inflammation, and a dose-dependent anti-inflammation was also observed⁽¹⁷⁾. These studies demonstrate the anti-inflammatory and anti-carcinogenic activities of γ -TmT in the colon.

CONCLUDING REMARKS

In collaboration with Dr. Nanjoo Suh, we demonstrated the dose-dependent inhibition of *N*-methyl-*N*-nitrosourea-induced mammary carcinogenesis in rats by 0.1, 0.3, and 0.5% of γ -TmT in the diet, and the inhibition was associated with the activation of PPAR- γ and the downregulation of estrogen signaling^(18, 19). In collaboration with Dr. Xi Zheng, we also showed the inhibition of LNCaP prostate cancer growth in a xenograft tumor model⁽²⁰⁾. The inhibition of prostate carcinogenesis in TRAMP mice by γ -TmT was reported by Barve *et al.*⁽²¹⁾. In the transgenic rat for adenocarcinoma of prostate (TRAP) model, γ -T, but not α -T, inhibited adenocarcinoma formation in the ventral lobe⁽²²⁾.

Overall, γ -TmT has been demonstrated to have broad cancer preventive activity. We have also shown that δ -T and γ -T are effective cancer preventive agents, whereas α -T is not. A common mechanism of action appears to be the trapping of RONS. In addition to trapping RONS, δ - and γ -T can be efficiently converted to side-chain metabolites, which retain the intact chromanol ring and may possess cancer preventive activities. Other mechanisms remain to be studied. When a high dose of α -T is used, it may decrease the blood and tissue levels of γ -T and diminish its cancer preventive activity^(7,8). Practical use of tocopherols for cancer prevention needs to be further studied.

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