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Cancer Prevention by Tocopherols and Tea Polyphenols

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Abstract

Tocopherols (vitamin E) and tea polyphenols have been reported to have cancer preventive activities. Large-scale human trials with high doses of alpha-tocopherol, however, have produced disappointing results. This review presents data showing that γ - and δ -tocopherols inhibit colon, lung, mammary and prostate carcinogenesis in animal models, whereas α -tocopherol is ineffective in animal and human studies. Possible mechanisms of action are discussed. A broad cancer preventive activity of green tea polyphenols has been demonstrated in animal models, and many mechanisms have been proposed. The cancer preventive activity of green tea in humans, however, has not been conclusively demonstrated and remains to be further investigated.

Keywords

tocopherols; vitamin E; green tea; polyphenols; cancer prevention

1. INTRODUCTION

Tocopherols are a group of fat-soluble compounds, collectively known as vitamin E, well known for their antioxidative action in protecting cell membrane against reactive lipid radicals [1]. Green tea polyphenols are not nutrients, but have been demonstrated to have antioxidative activities and beneficial health effects. Both groups of compounds have been studied by us and others for their cancer preventive activities in animal models and humans. A literature search on PubMed found 3,848 articles on “Vitamin E and cancer,” 859 articles on “Tocopherols and cancers” and 1,646 articles on “Green tea and cancer.” The mechanisms of these actions are rather complex, way beyond a simple “antioxidant” action. The results of human studies on these agents are even more complex and inconclusive.

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Scientists and the general public are facing massive amounts of, and sometimes confusing information. In this article, we will use mainly data from our laboratories to compare and contrast the actions of these two group of compounds with the purpose of critically analyzing the information to enhance the basic understanding of these topics. We plan to illustrate the importance of studying the activities of different forms of vitamin E as well as the bioavailability issues and redox properties of tea polyphenols. It also discusses the cautions needed to be applied in translating information from experimental systems *in vitro* to animal models and human populations. Directions for future research are also discussed.

2. STRUCTURES AND FUNCTION OF TOCOPHEROLS

Tocopherols, the major forms of vitamin E, are a family of fat-soluble phenolic compounds. Each tocopherol contains a chromanol ring system and a phytyl chain containing 16 carbons. Depending on the number and position of methyl groups on the ring, they exist as α -, β -, γ -, or δ -tocopherols (α -, β -, γ -, and 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 that can 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 has 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 captures lipid free radicals by one electron reduction. The resulting tocopherol phenoxyl radical can be reduced by ascorbic acid or glutathione to regenerate the tocopherol molecule. This is a well established physiological antioxidant mechanism for vitamin E to protect the integrity of biological membranes.

Tocopherols are widely occurring in dietary oils, such as corn, soybean, sesame and canola oils, as well as nuts. In these oils, γ -T is 3-5 times more abundant than α -T, and δ -T is also abundant in some oils. Upon ingestions, these tocopherols are transported to the liver via the lymphatic system. In the liver, the α -T transfer protein preferentially transfers α -T to very low-density lipoproteins, which carry tocopherols to the blood; γ -T is not effectively, and δ -T is even less effectively, transferred by this mechanism [1]. Therefore, α -T is the most abundant form of vitamin E in the blood and nonhepatic tissues; γ -T levels are lower and δ -T levels are even lower. The γ -T and δ -T in the liver, however, are actively metabolized through side-chain degradation by the ω -oxidation/ β -oxidation pathway [1]. Whereas the biological activities of α -T have been extensively studied, the cancer preventive activities of γ -T and δ -T are just beginning to be investigated.

3. TOCOPHEROLS AND CANCER IN HUMANS

Tocopherols, as effective antioxidants, have been proposed to protect against carcinogenesis; however, this proposal is supported by some epidemiological studies. 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 the risk of lung cancer [3]. In both studies, the protective effects were found in current smokers, suggesting a protective

effect of vitamin E against oxidative insults from cigarette smoking. In four case-control studies on lung cancer, three studies found lower serum α -T levels in lung cancer patients than in matched controls [3]. In a case-control study, Mahabir *et al.* 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 suggested that α -T accounts for 34-53% reduction in lung cancer risk [4]. Since the intake of γ -T was also increased in proportion to the α -T intake, the beneficial effect could also be due to the more abundant γ -T or the combined effects of all forms of tocopherols.

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 of all-rac- α -tocopheryl acetate or 200 μ g selenium (from L-selenomethionine) or both, daily for an average of 5.5 years, did not prevent prostate or other cancers [7]. In the recently published results on the follow-up (for 7-12 years) of this study, subjects receiving α -tocopheryl acetate had a hazard ratio of 1.17 for developing prostate cancers [8]. The headline in the new media that “vitamin E increases prostate cancer risk” is alarming. It was noted that, in the SELECT, the α -T supplement caused a 50% decrease in median plasma γ -T levels [7]. Since γ -T has been suggested to have strong anti-inflammatory and cancer preventive activities [3,9-13], the α -T-caused decrease in blood and tissue levels of γ -T may partially contribute to the increased prostate cancer risk. Another possible interpretation of the lack of cancer prevention effect of α -T is that supplementation of a nutrient to a population that is already adequate in this nutrient may not produce any beneficial effect. The exact reasons for these negative results from the SELECT and other trials are not known. Nevertheless, the disappointing outcome of these large-scale trials reflects our insufficient 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.

4. INHIBITION OF TUMORIGENESIS BY DIFFERENT FORMS OF TOCOPHEROLS IN ANIMAL MODELS

Previous cancer prevention studies in different animal models, mainly with 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 (named γ -TmT) [14-22]. 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 of these studies have been summarized in a recent commentary [23] and are discussed in the following sections.

4.1. Inhibition of lung tumorigenesis and tumor growth by γ -TmT, γ -T and δ -T

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 the 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 by 30%. 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). Treatment with the 0.3% γ -TmT diet during the carcinogen-treatment period, the post-initiation period, or the entire experimental period reduced the tumor multiplicity by 18%, 20%, or 30%, respectively. Moreover, the tumor burden was significantly reduced by γ -TmT treatment given during the tumor initiation period or the entire experimental period, by 36% and 43%, 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, without affecting apoptosis in non-tumorous lung tissues. 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 phospho-histone 2AX (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 mice and the levels were decreased by γ -TmT treatment. The anti-angiogenic activity of dietary γ -TmT was shown by the reduced microvessel density (CD31-labeled capillary clusters and blood vessels) in the peripheral area of the lung adenomas [14]. These results suggest the pro-apoptotic antioxidative, anti-inflammatory and anti-angiogenic activities of γ -TmT [14].

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), 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 those of 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% [14]. In a similar experiment, the effectiveness of different forms of 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. 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,

which were derived from NNK-induced lung tumors in A/J mice) in A/J mice by 54% and 80%, respectively, on Day 50 [16].

4.2. Inhibition of Colon Tumorigenesis by γ -TmT, γ -T and δ -T

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]. 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 the 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 the 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. In another study, the inhibitory activities of α -T, γ -T, δ -T and γ -TmT were compared in an AOM-induced colon carcinogenesis model in rats [22]. δ -T was the most effective in inhibiting the formation of aberrant crypt foci (ACF) and high grade dysplastic ACF. γ -TmT and γ -T had slightly lower activities, but α -T was ineffective [22]. This study is the first clear demonstration of the higher cancer preventive activity of δ -T than γ -T, and the ineffectiveness of α -T, in an animal carcinogenesis model.

4.3. Inhibition of mammary carcinogenesis by tocopherols

In previous studies on mammary carcinogenesis, four studies showed a protective effect of α -T, but one study showed no effect [3]. Recently, we demonstrated that dietary administration of 0.1%, 0.3% or 0.5% γ -TmT dose-dependently suppressed *N*-methyl-*N*-nitrosourea (NMU)-induced mammary tumor development and growth in rats [18,19]. The inhibitory action was associated with increased expression of p21, p27, peroxisome proliferator activated receptor- γ (PPAR- γ) and cleaved caspase-3; whereas protein kinase B (AKT) and the estrogen-dependent signaling pathways in mammary tumors were significantly decreased by γ -TmT treatment [19]. Furthermore, in NMU-treated rats, dietary γ -TmT, γ -T and δ -T decreased cell proliferation and increased apoptosis in these mammary tumors, but α -T was not effective [24].

4.4. Inhibition of prostate carcinogenesis by tocopherols

Previous studies on the effects of α -T and its synthetic analogs on prostate carcinogenesis and xenograft cancer growth have not yielded consistent results [3]. Recent work showed that 0.1% γ -TmT in the diet inhibited prostate carcinogenesis in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model [21]. During the development of prostate cancer in the TRAMP mouse, loss of nuclear factor-erythroid 2-related factor 2 (Nrf2) expression was observed, and γ -TmT treatment prevented the loss [21]. Takahashi *et*

al. demonstrated that γ -T (0.005 or 0.01% in the diet), but not α -T, decreased the number of adenocarcinomas in the ventral lobe in the transgenic rat for adenocarcinoma of prostate model [25], and the inhibitory action was associated with enhanced apoptosis (activation of caspases). We recently demonstrated the dose-dependent inhibition of LNCaP prostate cancer growth by γ -TmT (0.1%, 0.3% and 0.5% in the diet) in a xenograft tumor model in severe combined immunodeficiency (SCID) mice [20].

5. DOES VITAMIN E PREVENT OR PROMOTE CANCER?

We propose that, at the nutritional level, all forms of vitamin E are cancer preventive. This concept is consistent with many observations that the dietary intake or plasma levels of α -T and other tocopherols are inversely associated with cancer risk, especially among smokers [3,4,26,27]. At the supra-nutritional levels, however, α -T is not cancer preventive, which has been shown in several recent cancer prevention trials [5-8]. This concept is consistent with many studies in animal models, by others and us, showing the lack of cancer preventive activity of α -T supplementation [3,15,22]. Recent results further showed that δ -T, γ -T and γ -TmT are cancer preventive in animal models [3,14-24], and we propose that γ -T and δ -T are cancer preventive in humans as well [23]. This concept may help to explain the enhanced prostate cancer risk in subjects who took daily supplementation of 400 IU of α -T in the SELECT [8]. This supplementation caused a 50% decrease in the median plasma γ -T level [7], and this may decrease the cancer preventive effect of γ -T. High concentrations of α -T may also decrease the cancer preventive activity of γ -T or δ -T by competing for its binding to proteins that are important for cancer prevention, but this possibility remains to be demonstrated. A common mechanism of the cancer preventive action of tocopherols appears to be the trapping of RONS. In addition to trapping RONS, δ -T 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.

6. CHEMISTRY AND BIOCHEMISTRY OF TEA POLYPHENOLS

Tea, made from the leaves of the plant *Cadamellia sinensis*, is a popular beverage worldwide. By heating and drying the tea leaves, their characteristic constituents, catechins, are preserved. After brewing in hot water, about one-third of the materials are water extractable, of which 30–40% (by dry weight) are polyphenols, known as catechins. (–)-Epigallocatechin-3-gallate (EGCG) is the major catechin in tea, and (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epicatechin (EC) are also present in significant amounts [28,29]. The structure of EGCG is shown in Figure 2.

Black tea is produced by a process known as fermentation, in which the tea leaves are crushed to promote enzymatic oxidation and subsequent condensation of tea polyphenols, leading to the formation of oligomeric polyphenols (theaflavins) and polymeric polyphenols (thearubigins). Black tea contains 2–6% theaflavins, >20% thearubigins, and 3–10% catechins in the water-extractable materials. Tea leaves also contain 2–5% caffeine in the water-extractable material of green, oolong, and black tea.

Because of the polyphenolic structure, catechins are strong antioxidants by quenching reactive oxygen species (ROS) and by chelating metal ions to prevent the formation of ROS.

Catechins can also generate ROS via auto-oxidation in solution, such as in cell cultural medium. The phenolic groups are also donors for H-bonding; for example, EGCG has 8 phenolic groups serving as donors of H-bonding. The large sphere of hydration due to H-bonding and a molecular weight of 458 of EGCG limit its bioavailability [30]. The strong H-bonding ability also enables EGCG to bind many proteins with high affinity [31]. As reviewed previously, some of these proteins have been proposed as targets of EGCG for inhibitory actions against cancer cells [31]. For example, vimentin binds to EGCG with a K_d of 3.3 nM, and functional studies showed that EGCG inhibited the phosphorylation of vimentin at serine 50 and serine 55 with $IC_{50} = 17 \mu M$ [32]. The difference in effective concentrations is probably due to the nonspecific binding of EGCG to other proteins, which compete with the target protein. Binding of EGCG to the 67 kDa laminin receptor with a dissociation constant (K_d) value of $0.04 \mu M$ was observed using a surface plasmon resonance assay, and this was proposed to be a mechanism for the anti-cancer actions of EGCG [33]. A study with NMR spectroscopy showed the direct binding of tea polyphenols to the BH3 pocket of anti-apoptotic B-cell lymphoma-2 (Bcl-2) proteins with an inhibition constant (K_i) of 0.33–0.49 μM [34]. However, higher EGCG concentrations were needed to induce apoptosis [34]. A recent study demonstrated the binding of EGCG to both the WW and PPIase domains of peptidyl prolyl cis/trans isomerase (Pin 1) [35]. Biochemical studies showed a dissociation constant of 21.6 μM , and this was proposed as the mechanism for the cancer preventive activities of EGCG.

7. INHIBITION OF CARCINOGENESIS IN ANIMAL MODELS BY TEA POLYPHENOLS

In contrast to tocopherols, tea polyphenols are not nutrients. Some of the tea polyphenols, such as EGCG, have only limited bioavailabilities. Nevertheless, as reviewed previously [28,31], the evidence for the cancer preventive activities of green tea and its constituents in animal models is compelling. Most of the studies have been conducted with green tea and green tea polyphenols, especially the most abundant and biologically active EGCG.

7.1. Inhibition of lung tumorigenesis in rodent models

The inhibitory effects of tea preparations against lung tumorigenesis have been demonstrated in at least 20 studies [31,36]. Most studies used NNK or B[a]P to induce carcinogenesis. Oral administration of extracts or solutions of green tea, black tea, EGCG, EGC or theaflavins significantly decreased lung tumorigenesis in rats, mice, or hamsters [31,36]. Treatment of A/J mice with extracts of green or black tea also inhibited the spontaneous formation of lung tumors and rhabdomyosarcomas. In addition, oral administration of green tea infusion reduced the number of lung colonies of mouse Lewis lung carcinoma cells in a metastasis model.

Chung and coworkers demonstrated that caffeine effectively inhibited NNK-induced lung tumorigenesis in rats, and that the inhibitory effect of 2% black tea (containing 680 ppm caffeine) against lung tumorigenesis was due to caffeine [37]. This conclusion is different from the experiments with A/J mice, which demonstrated the inhibition of lung tumorigenesis by decaffeinated green and black tea preparations [38]. A possible

explanation of this difference is that the systemic bioavailability of tea polyphenols in mice is much higher than in rats [30]. In our recent study, administration of 0.5% Polyphenon E (PPE, a standardized green tea polyphenol preparation containing 65% EGCG, 25% other catechins, and 0.6% caffeine) or 0.044% caffeine in the drinking water, to tumor-bearing A/J mice (induced by a single dose of NNK administered 20 weeks earlier) for 32 weeks, inhibited the progression of lung adenomas to adenocarcinomas [39]. PPE and caffeine treatment inhibited cell proliferation, enhanced apoptosis, and decreased levels of c-JUN and phospho-extracellular regulated kinase1/2 (ERK1/2) in adenocarcinomas. These effects were not observed in the normal lung tissues, suggesting that the effect is selective against tumor cells. These results demonstrate the broad inhibitory activity of tea preparations in the inhibition of lung neoplasia at different stages of carcinogenesis.

For enhancing the cancer preventive activity of tea polyphenols, the synergistic inhibitory action of PPE in combination with a cholesterol-lowering agent, atorvastatin (trade name Lipitor), against NNK-induced lung carcinogenesis in A/J mice, has been demonstrated [40]. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. The synergistic action of this combination was also demonstrated in human lung cancer H1299 and H460 cells. In both the cell lines and the mouse lung tumors, downregulation of the anti-apoptotic proteins myeloid cell leukemia sequence 1 (MCL1) and B-cell lymphoma-extra large (BCL-X_L) and induction of apoptosis were associated with the synergistic inhibitory action [40]. It remains to be determined whether such synergistic actions between atorvastatin and tea polyphenols occur in humans.

7.2. Inhibition of tumorigenesis of the oral-digestive tract

Inhibitory effects of tea against tumorigenesis in the oral cavity, esophagus, stomach, small intestine, and colon have been shown in more than 30 studies [31,36]. For example, tea preparations were shown to inhibit 7,12-dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis in the hamster cheek pouch and *N*-nitrosomethylbenzylamine-induced rat esophageal carcinogenesis in our studies. EGCG also inhibited tumorigenesis in rat stomach and forestomach induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Black tea polyphenols (Polyphenon B), given at 0.05% in the diet, also effectively inhibited forestomach tumor formation; this inhibition was associated with increased apoptosis as well as reduced cell proliferation, inflammation and angiogenesis [31,36].

The inhibitory effects of tea and tea polyphenols against intestinal tumorigenesis have been consistently witnessed in mouse models [31,36]. For example, we observed that administration of EGCG at 0.02%-0.32% in drinking water dose-dependently inhibited spontaneous small intestinal tumorigenesis in *Apc*^{Min/+} mice, while caffeine did not show an inhibitory effect [41]. The inhibition was associated with increased levels of E-cadherin on the plasma membrane, as well as decreased levels of nuclear β -catenin, c-Myc, phospho-Akt, and phospho-ERK1/2 in tumors [41]. Administration of green tea extracts (0.6% in drinking fluid) also inhibited the formation of AOM-induced ACF in CF-1 mice on a high-fat diet [42]. Recently, Shimizu *et al.* [43] demonstrated the inhibition of AOM-induced ACF formation in male C57BL/KsJ-*db/db* mice by EGCG (0.01% and 0.1% in drinking water) through suppressing the activities of the insulin-like growth factor (IGF)/IGF-1

receptor (IGF-1R) axis. The elevated levels of IGF-1R, phospho-IGF-1R, phospho-glycogen synthase kinase-3 β (GSK-3 β) and β -catenin in the colonic mucosa were decreased by treatment with EGCG [43]. Also decreased were the plasma levels of IGF-1, insulin, triglyceride, cholesterol, and leptin.

In rat models, the effects of tea preparations on colon tumorigenesis have not been consistent [31,36]. This inconsistency in colon carcinogenesis is rather surprising, because the intestine is considered to be a promising site for chemoprevention with polyphenols that have low systemic bioavailability [30]. Orally ingested EGCG has only limited systemic bioavailability, with most of it passing through the colon; and the absorbed EGCG is excreted mostly through the bile into the intestine. Our recent animal study showed that, after the injection with AOM, the treatment of rats with PPE, 0.12 or 0.24% in the diet for 8 weeks, dose-dependently decreased the total number of ACF per rat. The inhibitory activity was associated with decreased levels of nuclear β -catenin and cyclin D1, and increased levels of retinoid X receptor α (RXR α), in the ACF with high-grade dysplasia [44]. After treatment with 0.24% PPE for 34 weeks, the incidence of adenocarcinoma decreased from 57% to 23%, and the multiplicity of adenocarcinoma and adenoma decreased by 80% and 45%, respectively (Yang, C.S. *et al.*, unpublished). The loss of RXR α expression was observed in colonic dysplastic ACF, adenomas, and adenocarcinoma, but the RXR α expression was (partially) retained in PPE treated rats in these lesions.

7.3. Inhibition of prostate tumorigenesis in mouse models

Administration of a green tea polyphenol infusion (0.1% in drinking water) to the TRAMP mice for 24 weeks markedly inhibited prostate cancer development and distant site metastases [45,46]. The inhibition was associated with increased apoptosis, decreased cell proliferation, decreased IGF-1 levels, and restored IGF binding protein 3 (IGF-BP3) levels in both serum and the dorso-lateral prostate [45,46]. These changes were associated with reduced levels of phosphatidylinositol 3-kinase (PI3K) as well as phosphorylated forms of AKT, ERK1 and ERK2. The treatment also significantly decreased levels of angiogenic and metastatic markers [46]. These results suggest that the inhibition of the IGF-1 signaling pathway, vascular endothelial growth factor A (VEGFA), and matrix metalloproteinases contribute to the cancer prevention activity of green tea polyphenols. Caporali *et al.* [47] reported similar inhibitory activities of orally administered green tea catechins on prostate tumor formation in the TRAMP model. The IGF-1 signaling pathway appears to be a key target for the inhibition; it is not clear whether tea polyphenols inhibit IGF-1 pathway by a direct or indirect action of tea polyphenols in the prostate or by systemic actions.

7.4. Inhibition of tumorigenesis in the mammary glands and other organs

There are at least 10 studies on possible inhibitory effects of tea against mammary tumorigenesis, but the results were inconsistent [31,36]. In our study, even at a high dose of 1,000 mg/kg b.w./day, *i.g.*, EGCG only slightly (non-significantly) reduced mammary tumor incidence and multiplicity in rats treated with NMU [48]. The lack of robust inhibition of mammary tumorigenesis [36] is likely to be due to low bioavailability of tea polyphenols in the mammary tissues. The observed low inhibitory effect of tea on mammary tumorigenesis may also be due to an indirect action of tea. For example, Rogers *et al.* [49] showed no

significant inhibitory effect of black tea administered during the promotion stage of DMBA-induced mammary tumorigenesis in rats maintained on the AIN76 diet. However, in rats on a high-fat diet, black tea was found to reduce the tumor number and size. The results suggest that black tea may decrease fat absorption and body fat levels, which subsequently influence estrogen metabolism and mammary tumorigenesis.

Green tea has shown to inhibit bladder carcinogenesis induced by *N*-(4-hydroxybutyl)-*N*-butyl-nitrosamine (OH-BBN) in rats [50,51]. In our study, PPE was administered (100 or 250 mg/kg b.w./day, intragastrically) to rats at 126 days of age (1 week after the final dose of OH-BBN of a total of 16 doses in 8 weeks). Palpable urinary bladder tumor incidence was reduced from 59% (control) to 40% (by 100 mg/kg) or 18% (by 250 mg/kg) [48].

Catechins are not the only cancer preventive constituents in tea; caffeine has also been shown to inhibit lung and skin carcinogenesis in mouse models [37,39,52]. The mechanism of action of caffeine in the inhibition of skin tumorigenesis has been thoroughly studied and discussed by Conney *et al* [52].

8. POSSIBLE MECHANISMS OF ACTION OF TEA POLYPHENOLS AND HUMAN RELEVANCE

Many mechanisms have been proposed for cancer prevention by tea constituents, and this subject has been reviewed recently [31]. For example, EGCG has been proposed to bind to different molecular targets, inhibit the activities of many key enzymes, and inhibit several receptor-dependent signaling pathways [31]. EGCG is known for its antioxidative actions, but it can also produce ROS, especially *in vitro* [53]. It is uncertain whether all of these proposed mechanisms are relevant to cancer prevention *in vivo*. Apparently, mechanisms derived from cancer prevention studies in animal models are likely to be more relevant. These include the induction of apoptosis in different animal models, inhibition of the phosphorylation of c-JUN and ERK1/2 in lung tumorigenesis model, suppression of phospho-AKT and nuclear β -catenin levels in colon cancer models, inhibition of the IGF/IGF-1R axis in colon and prostate cancer models, and suppression of VEGF-dependent angiogenesis in lung and prostate cancer models [31,39,41,43,46,47]. It is still unclear whether these molecules are direct targets for EGCG or downstream events of the primary action. In theory, *in vitro* experiments could provide more information on the detailed mechanisms. It is reasonable to assume that the high affinity binding proteins reported in the literature [31] could serve as initial targets, but this point remains to be substantiated in animal models. From the limited human studies that are available, action of tea constituents in reducing oxidative stress and enhancing the elimination of carcinogens could also be important [54,55].

In contrast to the strong evidence for the cancer preventive activity of tea constituents in animal models, results from epidemiological studies have not been consistent concerning the cancer preventive effect of tea consumption in humans [56]. The different results between animal and human studies is likely to be due to the lower quantities of human tea consumption as compared to the doses used in animal studies. In animal studies, the doses of tea preparations and the experimental conditions are set to maximize the opportunity to

detect a cancer prevention effect. In humans many life style, genetic polymorphism and other confounding factors reduce the power of epidemiological studies for detecting a cancer preventive effect. Smoking appears to be a strong confounding factor. For example, in a case-control study on the effect of green tea consumption on esophageal cancer in Shanghai by Gao *et al.* [57], a protective effect was only observed in non-smokers, mostly women. A recent systematic review of epidemiological studies in Japan on green tea consumption and gastric cancer indicated no overall preventive effect of green tea in cohort studies. However, a small consistent risk reduction was found in women, and the result was confirmed by pooling data of six cohort studies [58]. A possible explanation is that women were mostly non-smokers, whereas most men were smokers in this Japanese population. The relationship between tea consumption and cancer risk may become more clear if we quantify the tea consumption better, correct for confounding factors, and consider genetic polymorphisms of the populations.

9. CONCLUDING REMARKS

In this article, we have discussed two groups of phenolic compounds. Both of them are antioxidants, but their biological actions are way beyond their antioxidative activities. With tocopherols, we illustrated the importance of studying specific forms. We propose that at the nutritional level, all tocopherols are cancer preventive; either α -T or tocopherol mixtures contribute to cancer prevention. At the supra-nutritional levels, α -T is not effective in cancer preventive, based on human studies and some laboratory studies [3,5-8,15,22]. Our proposal that supra-nutritional levels of γ -T and δ -T are cancer preventive is based mainly on laboratory studies in animal models [3,14-24]. It would be important to conduct prevention trials with pure δ -T, γ -T or tocopherol mixtures that are rich in γ -T and δ -T to demonstrate their cancer preventive activities in human populations.

In studying tocopherols, tea polyphenols and other chemopreventive agents, cautions need to be applied in translating results from animal studies to human populations. Animal studies are usually designed to demonstrate the cancer prevention effects using doses higher than the levels of human consumption, without many of the confounding factors involved in human studies. Even more difficult is the use of information from cell line studies to predict cancer preventive activities in animals and humans. This is clearly seen in the case of the tea polyphenol EGCG, which has limited bioavailability and easily oxidized in cell culture medium to generate ROS. Such auto-oxidation is not like to occur *in vivo*. Therefore, predictions from cell line studies to *in vivo* situation could be problematic.

With the strong evidence provided by laboratory studies for the cancer preventive activities of tea constituents, even though the results from epidemiological studies are not conclusive, tea preparations can still be used for the prevention of certain types of human cancer. Results from laboratory studies discussed above could aid us to design the optimal conditions for cancer prevention trials and prospective studies as well as to accurately interpret results from epidemiological studies.

In future intervention studies with either tocopherols or tea polyphenols, it would be important to measure baseline blood levels of tocopherols or polyphenols as well as the

levels of the intervening agents at different time points of the trials. Individuals may absorb or eliminate these agents differently. These results may help to interpret the outcome of the trials. Individuals with low baseline levels of tocopherols may gain more benefit from the intervention with tocopherol, or even with tea polyphenols, if antioxidative action is a key mechanism of action.

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α -Tocopherol

$R_1 = R_2 = R_3 = \text{CH}_3$

β -Tocopherol

$R_1 = R_3 = \text{CH}_3, R_2 = \text{H}$

γ -Tocopherol

$R_2 = R_3 = \text{CH}_3, R_1 = \text{H}$

δ -Tocopherol

$R_1 = R_2 = \text{H}, R_3 = \text{CH}_3$

Figure 1.
Structures of tocopherols

Figure 2.
Structure of (-)-epigallocatechin-3-gallate (EGCG)