Published in final edited form as:

Cancer Lett. 2016 February 1; 371(1): 71–78. doi:10.1016/j.canlet.2015.11.010.

Dietary tocopherols inhibit PhIP-induced prostate carcinogenesis in CYP1A-humanized mice

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Abstract

Tocopherols, the major forms of vitamin E, exist as alpha-tocopherol (α -T), β -T, γ -T and δ -T. The cancer preventive activity of vitamin E is suggested by epidemiological studies, but recent large-scale cancer prevention trials with high dose of α -T yielded disappointing results. Our hypothesis that other forms of tocopherols have higher cancer preventive activities than α -T was tested, herein, in a novel prostate carcinogenesis model induced by 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP), a dietary carcinogen, in the CYP1A-humanized (hCYP1A) mice. Treatment of hCYP1A mice with PhIP (200mg/kg b.w., *i.g.*) induced high percentages of mouse prostatic intraepithelial neoplasia (mPIN), mainly in the dorsolateral glands. Supplementation with a γ -T-rich mixture of tocopherols (γ -TmT, 0.3% in diet) significantly inhibited the development of

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Conflicts of Interest:

No potential conflicts of interest were disclosed.

Conflict of interest statement

none

Authors' Contributions

Conception and design: JX Chen, GX Li, H Wang, CS Yang;

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Acquisition of data: JX Chen, GX Li, A Liu, MJ Lee;

Analysis and interpretation of data: JX Chen, GX Li, K Reuhl, CS Yang;

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Administrative, technical, or material support: N Suh, CS Yang;

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mPIN lesions and reduced PhIP-induced elevation of 8-oxo-deoxyguanosine, COX-2, nitrotyrosine, Ki-67 and p-AKT, and the loss of PTEN and Nrf2. Further studies with purified δ -T, γ -T or α -T (0.2% in diet) showed that δ -T was more effective than γ -T or α -T in preventing mPIN formations and p-AKT elevation. These results indicate that γ -TmT and δ -T could be effective preventive agents of prostate cancer.

Keywords

Tocopherols; prostate carcinogenesis; mPIN; PhIP; p-AKT

1. INTRODUCTION

Prostate cancer is one of the most commonly diagnosed malignancies and a leading cause of cancer-related death among men in the US and other developed countries (1, 2). Thus, developing preventive strategies to reduce prostate cancer incidence and severity is of profound importance. Several epidemiological studies have suggested an association between the consumption of red meat and risk of prostate cancer (3–5). One possible reason for this association is related to the presence of heterocyclic amines (HCAs), a group of potent chemical carcinogens that are found in meat cooked at high temperatures. 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), the most abundant HCA produced during high-temperature cooking of meat, has been implicated in numerous studies to elevate prostate cancer risk (6–9).

Our laboratory recently developed a PhIP-induced prostate carcinogenesis model in the CYP1A-humanized (hCYP1A) mice that resembles the early-stage of human prostate carcinogenesis (10). By replacing mouse Cyp1a2 (which mainly detoxifies PhIP) with human CYP1A2 (which preferentially activates PhIP), the hCYP1A mice administered with a single dose of PhIP was able to produce mouse prostatic intraepithelial neoplasia (mPIN), particularly in the dorsolateral prostate gland (DLG). Because murine dorsolateral prostate corresponds to the human prostate peripheral zone (11), the most common site for prostate cancer, the PhIP-induced prostate carcinogenesis in hCYP1A mice is a relevant model for studying human prostate cancer etiology and prevention.

Tocopherols, the major forms of vitamin E, are natural phenolic compounds widely found in nuts and vegetable oils such as corn, soybean and canola oils (12). Tocopherols exist as alpha-tocopherol (α -T), β -T, γ -T and δ -T, depending on the number and position of methyl group on the chromanol ring (Fig 1). Due to their ability to trap reactive oxygen and nitrogen species (RONS), tocopherols are important biological antioxidants, and their cancer preventive activities have been extensively studied (13–16). Epidemiological evidences have shown that higher dietary intake and blood levels of vitamin E are associated with lower risk for prostate cancer (15, 16). However, recent large-scale human prostate cancer trials with Vitamin E have yielded disappointing results. In the Physicians' Health Study II randomized control trial and the Selenium and Vitamin E Cancer Prevention Trial (SELECT), supplementation with high doses of α -T failed to reduce the risk of prostate or other cancers (17, 18). It is possible that α -T inhibits carcinogenesis only in those who have a low vitamin E status at baseline. Another possibility is that the other vitamin E forms, such as γ -T and δ -

T, in our diet have much stronger cancer preventive activity than α -T. Indeed, recent studies have shown that a γ -T-rich mixture of tocopherols (γ -TmT), which contains 56.8% γ -T, 24.3% δ -T, 13.0% α -T and 1.5% β -T, inhibited the development of prostate cancers in the TRAMP mice as well as lung, colon and mammary cancers in rodent models (19–25). Purified γ -T also demonstrated strong inhibitory effects on the development of palpable prostate tumors and mPIN lesions in a rat model (26).

In the present study, we investigated the effects of different preparations of tocopherols on PhIP-induced prostate carcinogenesis. The dosages of γ -TmT (0.3% in diet) or δ -T, γ -T and α -T (0.2% in diet) were selected from the optimal doses observed in previous studies and correspond to the intake of 1.5g or 1.0g of tocopherols, respectively, for a person consuming 500g of diet per day (20–22, 27). These doses, although higher than the nutritional level, have been reported to be nontoxic (28). Dietary γ -TmT and δ -T were found to inhibit the development of mPIN lesions and related molecular events, while γ -T and α -T were less effective. The results suggest that the inhibitory effect of tocopherols involves the reduction in cellular oxidative and nitrosative stress and inhibition of the PI3K/AKT signaling pathway.

2. MATERIALS AND METHODS

2.1 Reagents & animal studies

γ -TmT was obtained from Cognis Corporation (Kankakee, IL). δ -T, γ -T and α -T were purified from commercial tocopherol preparations to 97% purity by automated flash chromatography as previously described (27). PhIP was purchased from Wako Pure Chemical Industries (Osaka, Japan) and dissolved in deionized water to 20 mg/ml concentration before administration. The hCYP1A mice from Jackson Laboratories (Bar Harbor, ME) were bred in our animal facility as previously described (10). All animal studies were conducted in accordance to the Institutional Animal Care and Use protocol (Number 02-027) of Rutgers University. Starting at 5–6 weeks of age, male hCYP1A mice were placed on the AIN93M diet (as control) or AIN93M supplemented with 0.3% γ -TmT or 0.2% purified tocopherol diet (prepared by Research Diets, New Brunswick, NJ) for one week before intragastric administration of PhIP (200 mg/kg b.w.) or water (untreated group). All diets were stored in sealed plastic bags in boxes at around -4°C and warmed to room temperature before given to mice fresh on a weekly basis. Mice were maintained at room temperature ($20 \pm 2^{\circ}\text{C}$) with a relative humidity of $50 \pm 10\%$ and alternating 12 h light/dark cycle. Water and diet were provided *ad libitum*. Body weight and food consumption were monitored weekly. At 40 weeks after PhIP treatment, mice were euthanized by CO_2 asphyxiation. Blood was taken by cardiac puncture for serum extraction. Prostate was excised as a whole and fixed in 10% buffered formalin before transferring to 70% ethanol.

2.2 Histopathological analysis

After formalin fixation, the whole prostate was embedded in paraffin wax and serially sectioned at 4 μm . Histopathological analysis of the dorsolateral prostate glands was conducted on 3 step sections stained with hematoxylin and eosin. The mPIN lesions in the DLGs were identified according to consensus criteria established by a panel of human and

veterinary pathologist with expertise in prostate cancer, which are generally used to score prostate lesions in different transgenic mouse models (29, 30). The mPIN lesion is characterized by proliferation of epithelial cells within pre-existing glandular spaces that display cellular pleomorphism and nuclear atypia such as nuclear enlargement, nuclear hyperchromasia, chromatin clumping, and/or prominent nucleoli. Proliferation is demonstrated by stratification of epithelial cells and can acquire tufting, micropapillary, or cribriform growth patterns. Inflammation, as identified by peri- or intra-epithelial infiltration of any inflammatory cells (i.e., neutrophilic and/or mononuclear), was also assessed.

2.3 Immunohistochemistry and quantification

Immunohistochemical analysis was carried out using a standard avidin–biotin peroxidase complex method. Antibodies used in the study include: anti-8-oxo-dG (mouse monoclonal; JalCA), anti-nitrotyrosine (rabbit polyclonal; Millipore), anti-COX-2 (M-19; goat polyclonal; Santa Cruz), anti-Nrf2 (rabbit polyclonal; Santa Cruz), anti-Ki-67 (rabbit polyclonal; Abcam), anti-p-AKT (rabbit polyclonal; Abcam), and anti-PTEN (rabbit monoclonal; Cell Signaling Technology). Sections were then counterstained with hematoxylin. The total number of cells, the number of positively stained cells (including 1+, 2+, and 3+), and the immunostaining intensity were quantified using the Aperio ScanScope GL system (Vista, CA).

2.4 Tocopherols and metabolites analysis

For the analysis of serum tocopherols and metabolites levels, a previous described method was used (27). In brief, the serum sample was extracted with hexane and then analyzed by HPLC. For the analysis of tocopherol metabolites, samples were de-conjugated by β -glucuronidase and sulfatase before extraction and analysis by HPLC.

2.5 Statistical analysis

Results were analyzed using the GraphPad Prism software (GraphPad, CA). Student t-test was used to determine the differences in 2 groups. One-way ANOVA followed by Tukey's post hoc test was used to compare multiple groups. Differences were considered statistically significant when $p < 0.05$.

3. RESULTS

3.1 Inhibitory effect of dietary γ -TmT on PhIP-induced mPIN lesions in hCYP1A mice

Our previous study demonstrated that PhIP treatment induced a high percentage of mPIN lesions in the dorsolateral prostate of hCYP1A mice after 40 weeks (10). Examination of multiple mouse prostate cancer models over the years has shown that mPIN represent pre-invasive neoplasia of the prostate and such lesions often progress to invasive carcinoma (29). To study the effect of γ -TmT on the PhIP-induced prostate carcinogenesis, hCYP1A mice were fed the AIN93M diet (as control) or 0.3% γ -TmT-supplemented diet from one week before PhIP administration until the mice were sacrificed after 40 weeks. After an initial 5–10% drop in body weight in the PhIP-treated hCYP1A mice, the mice appeared in good health and no significant difference in body weight was found between animals in the control and γ -TmT-supplemented groups at week 40 (data not shown).

Histopathological analysis of the DLGs showed that PhIP-treated mice on the control diet exhibited large papillary lesions of stratified epithelial cells protruding into the glandular lumen of the dorsal (Fig. 2A–C) and lateral prostate glands (Fig. 2D–F). The neoplastic cells frequently displayed cellular pleomorphism and marked nuclear atypia (i.e., enlarged nuclei, prominent nucleoli, and nuclear hyperchromasia). These features are consistent with characteristics of mPIN lesions as classified by Shappell *et al.* and Ittmann *et al.* (29, 30). Less advanced mPIN with smaller papillary foci and/or milder nuclear atypia were identified in the γ -TmT-supplemented mice treated with PhIP in both dorsal (Fig. 2G–I) and lateral prostate glands (Fig. 2J–L). The effect of the γ -TmT diet on the development of mPIN lesions are summarized in Table 1. With an average of 75–90 DLGs analyzed per prostate, mice on the γ -TmT-supplemented diet showed ~65% reduction in the percentage of glands with mPIN lesions compared to the control diet group ($p < 0.001$), indicating a preventive effect of dietary γ -TmT on the development of mPIN. On the other hand, γ -TmT supplementation did not significantly affect the severity or frequency of inflammatory infiltrate observed in the DLGs of PhIP-treated mice (Table 1). While minor intra-epithelial infiltration of mononuclear and neutrophilic inflammatory cells was frequently observed in the ventral glands (data not shown), mild peri-epithelial infiltration of mononuclear inflammatory cells was found in 5/14 mice on the control diet and 4/14 mice on the γ -TmT-supplemented diet.

3.2 Dietary γ -TmT modulates PhIP-induced changes in 8-oxo-dG, COX-2, nitrotyrosine, Nrf2 and Ki-67 in the DLGs

There is growing evidence that oxidative stress contributes to human prostatic carcinogenesis (31–33). In PhIP-treated hCYP1A mice, oxidative stress was markedly increased in the DLGs, suggesting it plays a role in the prostate carcinogenesis (10). To determine whether PhIP-induced oxidative stress is reduced by tocopherols, we measured the levels of 8-oxo-dG, a common marker of oxidative DNA damage, and the expression of COX-2, a cyclooxygenase that catalyzes the formation of pro-inflammatory prostaglandins. In untreated mice, a very low basal level of nuclear 8-oxo-dG and COX-2 staining was observed in the epithelial cells of DLGs (Fig. 3A and E). In PhIP-treated mice on the control diet, strong nuclear stainings were detected for both 8-oxo-dG and COX-2 (with more positive-staining in mPIN lesions than in apparently normal glands) (Fig. 3B and F). However, reduced numbers of positive-stained cells were found in the mice given the γ -TmT diet, (Fig. 3C and G). Quantification of the immunostaining indicated that dietary γ -TmT dramatically decreased the percent of 8-oxo-dG and COX-2 positive-stained cells, but their levels were still higher than those of the untreated mice (Fig. 3D and H).

We also investigated nitrotyrosine, a known marker of nitrosative stress that arose from reactive nitrogen species nitrating tyrosine residues. In the untreated hCYP1A mice, a low level of nitrotyrosine staining was observed in the DLGs (Fig. 3I). The nitrotyrosine-positive staining increased markedly in the DLGs (with more staining in mPIN lesions than in apparently normal glands) of PhIP-treated mice on the control diet, but this increase was reduced in mice given the γ -TmT diet (Fig. 3J and K). Quantification of the immunostaining showed that dietary γ -TmT significantly decreased nitrotyrosine positive-stained cells, but the level remained higher than the untreated mice (Fig. 3L).

Nrf2 is a transcription factor that regulates the expression of many cytoprotective enzymes and proteins. In the DLGs of untreated mice, a high level of nuclear and cytoplasmic Nrf2 staining was observed (Fig. 3M). In PhIP-treated mice on the control diet, Nrf2 staining was greatly reduced in the DLGs (similar weak staining in mPIN lesions as in apparently normal glands), and the reduction was partially prevented in mice on the γ -TmT diet (Fig. 3N and O). Immunostaining quantification indicated that dietary γ -TmT significantly prevented the decrease of Nrf2 positive-stained cells (Fig. 3P).

Lastly, Ki-67, a well-established marker of cell proliferation, was used to examine the effect of γ -TmT. In the untreated hCYP1A mice, a minute basal level of Ki-67-positive cells was observed in the DLGs (Fig. 3Q). In PhIP-treated mice on the control diet, increased Ki-67-positive staining was detected in the mPIN lesions, but a lower number of Ki-67-positive cells were found in mPINs of mice given the γ -TmT diet (Fig. 3R and S). Quantification of the immunostaining showed that dietary γ -TmT significantly lowered the percent of Ki-67-positive cells in the DLGs (Fig. 3T).

3.3 Dietary γ -TmT prevents PhIP-induced dysregulation of p-AKT and PTEN in the DLGs

The PTEN/PI3K/Akt signalling pathway is a critical pathway for cell proliferation, cell cycle progression, and survival. One possible molecular mechanism of PhIP-induced prostate carcinogenesis in hCYP1A is the activation of AKT in response to cellular oxidative stress and the loss of PTEN, which is believed to be a major sequence of events in human prostate cancer (10, 34, 35). To determine the effect of dietary γ -TmT on PhIP-induced activation of the PTEN/PI3K/Akt pathway, we assessed the protein levels of both activated p-AKT and PTEN. In the DLGs of untreated mice, a low basal level of p-AKT staining was observed (Fig. 4A). In PhIP-treated mice on the control diet, p-AKT staining was elevated (with more positive-staining in mPIN lesions than in apparently normal glands), but elevation was diminished in mice on the γ -TmT diet (Fig. 4B and C). Quantitative analysis of the immunostaining indicated that dietary γ -TmT significantly attenuated the percentage of p-AKT positive-stained cells to the basal level (Fig. 4D). PTEN, a phosphatidylinositol phosphatase with tumor suppressor activity, was expressed at high levels in the DLGs of untreated mice (Fig. 4E). In PhIP-treated mice on the control diet, PTEN staining was greatly reduced (with less staining in mPIN lesions than in apparently normal glands), but this reduction was partially prevented in mice on the γ -TmT diet (Fig. 4F and G). Quantitative immunostaining analysis showed that dietary γ -TmT dramatically increased the number of PTEN positive-stained cells to a level comparable to untreated mice (Fig. 4H).

3.4 Dietary δ -T, γ -T and α -T inhibits PhIP-induced prostate carcinogenesis

We also investigated the effect of purified δ -T, γ -T and α -T on PhIP-induced prostate carcinogenesis model. The hCYP1A mice were given AIN93M (as control) or 0.2% δ -T, γ -T, or α -T supplemented diet starting at one week before PhIP administration until sacrifice after 40 weeks. The results on the development of mPIN lesions are summarized in Table 1. With 99-111 DLGs analyzed per prostate, mice on all of the tocopherol-supplemented diets had a significantly lower percentage of glands with mPIN than mice on control diet ($p < 0.05$). Mice on the δ -T diet displayed ~60% reduction in mPIN lesions, greater than

~23% reduction observed in mice on the γ -T or α -T diets. Quantitative analysis of p-AKT IHC staining showed that elevation of p-AKT in the DLGs of PhIP-treated mice (with more positive-staining in mPIN lesions than in apparently normal glands), and it was significantly reduced by δ -T and moderately attenuated by γ -T and α -T (Fig. 5). On the other hand, purified tocopherol supplementation did not have significant effect on the infiltration of inflammatory cells in the DLGs of PhIP-treated mice (Table 1).

3.5 Dietary tocopherols increase serum tocopherols and metabolites levels

The serum concentrations of δ -T, γ -T, α -T and their short-chain degradation metabolites, CEHC and CMBHC, in the above experiments are shown in Table 2. In mice on the γ -TmT diet, the level of all three tocopherols increased significantly. While α -T was the highest and increased in greatest value, its relative increases (~1-fold) was much lower than that of δ -T (~13-fold) or γ -T (~25-fold). Also, even with the low concentration of δ -T (0.80 μ M) and γ -T (1.74 μ M), the levels of the major short-chain degradation metabolites, CEHC and CMBHC, were much higher (~3 μ M for both). In mice on the purified tocopherols supplemented diet, increases in the respective form of tocopherol and their CEHC and CMBHC metabolites were observed. The pattern of changes due to the supplementation was similar to that produced by γ -TmT. However, the serum level of δ -T in the δ -T-supplemented group was lower than expected, possible due to its extensive side-chain degradation metabolism to a higher level of δ -CEHC and δ -CMBHC. The prostate levels of the tocopherols and metabolite were not measured because the tissues were used for histological analysis.

4. DISCUSSION

The present study demonstrated that dietary supplementation with γ -TmT and δ -T significantly reduced the formation and severity of mPIN lesions in a prostate carcinogenesis model induced by PhIP dietary carcinogen in CYP1A-humanized mice. Moderate inhibitory activity was also observed with γ -T and α -T supplementations. To our knowledge, this is the first demonstration of the prostate cancer preventive effects of purified δ -T and γ -TmT in a dietary carcinogen-induced prostate cancer model.

Strong evidences support the notion that cellular and oxidative stress play an important role in prostate carcinogenesis (31, 32). Our results suggest the reduction of cellular oxidative and nitrosative stress by tocopherols is prominently involved in the inhibition of the PhIP-induced mPIN lesions. In the PhIP-treated hCYP1A mice, we observed highly elevated 8-oxo-dG and nitrotyrosine levels and COX-2 expression as well as decreased Nrf2 expression in the mPIN lesions, possibly due to PhIP-induced oxidative stress, epithelial cell proliferation and associated inflammation observed in the present and previous studies (10). The dietary tocopherol mixture was able to prevent these molecular changes and reduced cell proliferation, especially in the mPIN lesions. The reduction of 8-oxo-dG and nitrotyrosine by tocopherols is primary due to the ability of tocopherols and their side-chain metabolites (with intact chromanol ring) to trap RONS. The prevention of COX-2 elevation could be due to the anti-inflammation effect of tocopherols due to trapping of RONS and the production of long-chain degradation metabolites of δ -T and γ -T, which inhibit COX-2 activity (13). The exact mechanism in which γ -TmT prevented the decrease of Nrf2 or the

increase of Ki-67 is unclear, although it may be attributed to the less advanced mPIN lesions and smaller papillary foci found in the γ -TmT-supplemented mice.

PTEN/PI3K/Akt is a critical cellular pathway, in which dysregulation has been widely documented in human prostate cancer and cancer cell lines (35–37). In our study, γ -TmT was found to significantly attenuate PhIP-induced elevation of p-AKT and loss of PTEN. To our knowledge, this is the first demonstration of inhibitory activity of tocopherols on p-AKT and PTEN in an animal model of prostate cancer. While the exact mechanism of inhibition remains to be further investigated, studies have shown that excess oxidative stress can stimulate the PI3K/Akt pathway (34, 38), suggesting that antioxidant activities of tocopherol may be responsible for the attenuation of p-AKT elevation and PTEN loss. In addition, recent results from our laboratory demonstrated that δ -T is an effective inhibitor of the activation of AKT in several prostate cancer cell lines, possibly by preventing internalization of receptor tyrosine kinases that phosphorylate AKT (Wang et al., unpublished results). Also, γ -T at high concentrations has been demonstrated to recruit PHLPP phosphatases to dephosphorylate p-AKT in cell lines (39).

Our study with purified δ -T, γ -T, or α -T revealed that δ -T is more effective in preventing mPIN formation and inhibiting p-AKT elevation than γ -T or α -T. The superior chemopreventive effect of δ -T over the other forms is consistent with previous studies in azoxymethane-induced colon carcinogenesis model in rats, the growth of human lung cancer cells in nude mice, and colon cancer cell lines in culture (23, 27, 40). However, γ -T was found to have similar efficacy as δ -T in inhibiting N-methyl-N-nitrosourea-induced hormone-dependent mammary tumorigenesis in rats (41). The modest inhibiting effect of α -T on development of mPINs lesions and p-AKT elevation was unexpected and does not conform to its ineffectiveness in previous studies comparing different purified tocopherols in model systems (23, 26, 27, 41). Since the PhIP-induced prostate cancer model represents a model of early-stage prostate carcinogenesis, it is possible that the antioxidant properties of α -T may exert a preventive effect on the development of preinvasive neoplastic lesions.

The remarkable increase of short-chain metabolites in mice on the γ -TmT are consistent with the general understanding that after tocopherols are absorbed into the liver, α -T is effectively transferred by α -T transfer protein to the blood through very low-density lipoproteins, whereas γ -T and δ -T are less effectively transferred by this mechanism, but are largely metabolized through side-chain degradation via β -oxidation pathway in the liver (14). Although not measured in our study, long-chain degradation metabolites of δ -T and γ -T have been shown to be produced and biologically active (13). The abundant CEHCs and CMBHCs, with their intact chromanol ring, are likely to possess some of the activities of their parent tocopherols. The possible contribution of these side-chain degradation products in the observed chemopreventive effect will need to be further investigated.

In summary, we demonstrated the inhibitory effects of γ -TmT and purified δ -T, γ -T and α -T in a novel PhIP-induced early stage prostate carcinogenesis model. This inhibition is associated with significant reductions in cellular oxidative and nitrosative stress and inhibition of the PI3K/Akt signaling pathway. Our results cannot explain the lack of prostate cancer preventive action of α -T in previous human trials. Nevertheless, the strong cancer

preventive activities of γ -TmT and δ -T suggest that these tocopherols should be further investigated in preclinical and clinical studies.

Acknowledgments

Funding

This work was supported by the US NIH grants (RO1 CA133021 & RO1 AT007036) and the John L. Colaizzi Chair endowment as well as shared facilities funded by NCI cancer center support grant (CA72720) and NIEHS center grant (ES05022). JX Chen was supported by NIEHS training grant (T32ES007148) and NIH fellowship grant (F31CA168333).

The authors thank Yu-Hai Sun for her technical assistance in the preparation of histologic slides and the Laboratory of Animal Service at the Department of Chemical Biology for animals care.

Abbreviations

HCA s	heterocyclic amines
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
hCYP1A mice	cytochrome P450 1A-humanized mice
mPIN	mouse prostate intraepithelial neoplasia
DLG s	dorso-lateral glands
γ-TmT	a γ -T rich mixture of tocopherols
α-T	alpha-tocopherol
γ-T	gamma-tocopherol
δ-T	delta-tocopherol
RONS	reactive oxygen and nitrogen species
8-oxo-dG	8-oxo-deoxyguanosine
Nrf2	nuclear factor erythroid 2-related factor 2
COX-2	cyclooxygenase-2
p-AKT	phospho-AKT
PTEN	phosphatase and tensin homolog
CEHC	carboxyethyl hydroxychroman
CMBHC	carboxymethylbutyl hydroxychroman

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Highlights

- Dietary γ -TmT inhibits PhIP-induced prostate mPIN lesion in CYP1A-humanized mice
- γ -TmT reduces PhIP-induced oxidative and nitrosative stress in the mouse prostate
- γ -TmT prevents PhIP-induced dysregulation of p-AKT and PTEN
- δ -T is more effective than γ -T or α -T in inhibiting PhIP-induced mPINs and p-AKT

Figure 1.

Structures of α , β , γ , and δ -tocopherols. α -T is 5, 7, 8-trimethylated, whereas β -T is 5, 8-dimethylated, γ -T is 7, 8-dimethylated and δ -T is 8-methylated.

Figure 2.

PhIP-induced PIN in the DLGs and effects of dietary γ -TmT. A and D, normal dorsal and lateral prostate glands of untreated mice on control diet. B and E, dorsal and lateral prostate glands with mPIN lesion from PhIP-treated mice on control diet. C and F, respective high magnification images of B and E with marked nuclear atypia (arrowheads). G and J, normal dorsal and lateral prostate glands of untreated mice on 0.3% γ -TmT diet. H and K, dorsal and lateral prostate glands with less advanced mPIN lesions from PhIP-treated mice on 0.3% γ -TmT diet. I and L, respective high magnification images of the H and K with milder nuclear atypia (arrowheads). All DLGs were taken from hCYP1A mice at Week 40. Scale bars represents 50 μ m.

Figure 3.

Dietary γ -TmT modulates PhIP-induced changes in 8-oxo-dG, COX-2, nitrotyrosine, Ki-67 and Nrf2 in the DLGs of hCYP1A mice. Lateral glands are used as representative: A, E, I, M and Q, normal DLGs from untreated mice on control diet showing low basal levels of 8-oxo-dG (~1.7%), COX-2 (~3.1%), nitrotyrosine (4.3%), and Ki-67 (0.1%) and high basal level of Nrf2 (~74.2%) immunostaining. B, F, J, N and R, mPIN lesions from PhIP-treated mice on control diet showing elevated 8-oxo-dG (~60.0%), COX-2 (~92.2%), nitrotyrosine (~50.3%), and Ki-67 (~1.9%) and reduced Nrf2 (~23.0%) immunostainings. C, G, K, O and S, mPIN lesions from PhIP-treated mice on 0.3% γ -TmT diet showing decreased 8-oxo-dG (~7.9%), COX-2 (~38.3%), nitrotyrosine (~24.3%), and Ki-67 (~1.0%) and increased Nrf2 (~56.1%) immunostainings. D, H, L, P and T, quantitative analysis of the 8-oxo-dG, COX-2, nitrotyrosine, Ki-67 and Nrf2 immunostainings in the DLGs, respectively (n = 4–5) (ANOVA-Tukey, $p < 0.05$). Untreated (U); Scale bars represents 50 μ m.

Figure 4.

Dietary γ -TmT prevents PhIP-induced dysregulation of p-AKT and PTEN in the DLGs of hCYP1A mice. Lateral glands are used as representative: A and E, DLGs from untreated mice on control diet showing low basal level of p-AKT (~1.3%) and high basal level of PTEN (~63.1%) immunostainings. B and F, mPIN lesions from PhIP-treated mice on the control diet showing elevated p-AKT (~12.4%) and reduced PTEN (~4.5%) immunostainings. C and G, mPIN lesions from PhIP-treated mice on 0.3% γ -TmT diet showing decreased p-AKT (~0.9%) and increased PTEN (~40.0%) immunostainings. D and H, quantitative analysis of the p-AKT and PTEN immunostaining in the DLGs, respectively (n = 4–5) (ANOVA-Tukey, $p < 0.05$). Untreated (U); Scale bars represents 50 μ m.

Figure 5.

Inhibitory effects of purified tocopherols on p-AKT in the DLGs of PhIP-treated hCYP1A mice. Lateral glands are used as representative: A, normal DLGs from untreated mice on control diet showing lack of p-AKT (~1.0%) immunostaining. B, representative mPIN lesion from PhIP-treated mice on the control diet showing high level of p-AKT (~15.9%) immunostaining. C, D and E, mPIN lesions from PhIP-treated mice on 0.2% δ -T, γ -T and α -T diet, respectively, showing reduced levels of p-AKT (~3.5%, ~7.7%, ~9.8%, respectively) immunostaining. F, quantitative analysis of the immunostaining in the DLGs (n = 4–5) (ANOVA-Tukey, $p < 0.05$). Untreated (U); Scale bars represents 50 μ m.

Table 1
Inhibitory effect of γ -TmT and purified tocopherols on PhIP-induced mPIN lesions in hCYP1A mice

Group (#mice)	Diet	PhIP (mg/kg, i.g.)	DLGs analyzed per animal (#) [†]	Glands with mPIN lesion (%) [†]	Inflammatory infiltrates in DLGs
hCYP1A (n=5)	AIN93M	-	75 ± 18	0	0/5
hCYP1A (n=14)	AIN93M	200	81 ± 21	30.9 ± 12.9	5/14
hCYP1A (n=14)	0.3% γ -TmT	200	90 ± 33	10.9 ± 6.7 [*]	4/14
hCYP1A (n=21)	AIN93M	200	108 ± 48	27.2 ± 6.8 ^a	7/21
hCYP1A (n=13)	0.2% δ -T	200	105 ± 51	10.8 ± 3.6 ^b	4/13
hCYP1A (n=12)	0.2% γ -T	200	99 ± 42	21.0 ± 6.4 ^c	3/12
hCYP1A (n=11)	0.2% α -T	200	111 ± 48	21.1 ± 5.8 ^c	4/11

[†] Value expressed as mean ± SD.

^{*} Significantly decreased from the AIN93M control diet group (Student t-test, $p < 0.001$).

^{a,b,c} The differences between groups with different letters were statistically significant (ANOVA-Tukey's test, $p < 0.05$).

Table 2

Serum levels of tocopherols and metabolites in PhIP-treated hCYP1A mice at Week 40

Diet Group (n=4)	α -T	γ -T	δ -T	α -CEHC	γ -CEHC	δ -CEHC	α -CMBHC	γ -CMBHC	δ -CMBHC
Control	12.69±2.76	0.07±0.02	0.06±0.00	0.17±0.06	0.21±0.12	0.03±0.01	0.01±0.01	0.10±0.09	0.06±0.04
0.3% γ -TmT	26.56±8.12*	1.74±0.67*	0.80±0.48*	0.19±0.08	3.12±1.07*	2.57±1.48*	0.17±0.10*	3.96±2.56*	3.98±2.33*
Control	13.82±3.24	0.39±0.09	0.08±0.01	0.10±0.04	0.42±0.38	0.53±0.20	0.09±0.09	0.01±0.03	0.02±0.03
0.2% δ -T	13.44±3.99	0.44±0.13	0.54±0.07*	0.06±0.06	0.10±0.12	7.89±1.56*	0.04±0.08	0.00±0.00	8.21±2.77*
0.2% γ -T	11.50±4.79	2.57±1.66*	0.07±0.02	0.08±0.06	5.09±0.85*	0.44±0.36	0.06±0.05	2.08±0.77*	0.11±0.12
0.2% α -T	38.66±2.67*	0.57±0.18	0.07±0.01	0.17±0.09	0.26±0.26	0.31±0.22	0.16±0.08	0.02±0.05	0.04±0.06

* Significantly increased from the respective AIN93M control diet group (Student t-test, $p < 0.05$). Values expressed as mean $\pm$ SD (μ M).