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# Lack of Effect of Dietary α-Tocopherol on Chemically Induced Hepatocarcinogenesis in Rats

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***Abstract:*** *We investigated the effects of α-tocopherol on diethylnitrosamine (DEN) initiation-phenobarbital (PB) promotion of hepatic foci in female Sprague-Dawley rats. Groups of eight rats were initiated with DEN (15 mg/kg) at 24 hours of age. After weaning, they received diets containing 500 ppm PB and various concentrations of α-tocopherol, deficient (0 ppm), adequate (100 ppm), and supplemented (5,000 ppm), for 24 weeks. Rats fed α-tocopherol-supplemented diets had significantly greater hepatic α-tocopherol levels than those fed α-tocopherol-deficient or -adequate diets ($p < 0.05$). Liver lipid peroxidation (measured as thiobarbituric acid-reactive substances) was significantly greater in rats fed α-tocopherol-deficient diets than in those fed α-tocopherol-adequate or -supplemented diets ($p < 0.05$). The dietary α-tocopherol level had no significant effect on the ratios of reduced glutathione (GSH) to oxidized GSH or reduced GSH to total GSH in the liver or on the plasma prostaglandin $E_2$ concentration or on the activities of hepatic cytosolic and particulate protein kinase C. Rats fed α-tocopherol-adequate or -supplemented diets had significantly greater hepatic glutathione S-transferase, GSH reductase, and GSH peroxidase activities than those fed α-tocopherol-deficient diets ($p < 0.05$). The dietary α-tocopherol level did not significantly affect the formation of hepatic γ-glutamyl transpeptidase- and placental glutathione S-transferase-positive foci. These results suggest that α-tocopherol does not influence hepatic foci formation and that reactive oxygen species may not be the underlying mechanism of hepatic foci formation in this DEN initiation-PB promotion model of hepatocarcinogenesis.*

## Introduction

Generation and accumulation of reactive oxygen species (ROS) have been shown to cause damage to DNA bases, and this plays a significant role in the multistage process of carcinogenesis that includes initiation and promotion (1). In addition, ROS can modify intercellular communication, protein kinase activity, membrane structure and function, and gene expression and result in modulation of cell growth (2). Removal and inactivation of ROS are dependent on the antioxidative defense system. The antioxidative capacity is determined by dynamic interactions between individual antioxidants, including vitamins A, E, and C, β-carotene, reduced glutathione (GSH), and several antioxidative enzymes (3). GSH peroxidases, GSH reductase, and GSH transferases are among the principal antioxidant enzymes (4). GSH peroxidases catalyze the decomposition of $H_2O_2$ to $H_2O$ and reduce organic peroxides to their corresponding alcohols (5), whereas GSH reductase regenerates GSH from glutathione disulfide (GSSG). GSH transferases decompose lipid hydroperoxides. Antioxidants have been reported to protect against cancer (6). α-Tocopherol is known to have the greatest biological activity of the various stereoisomers of vitamin E (7). *In vivo*, α-tocopherol is the most abundant lipid-soluble antioxidant (8) and acts as an important inhibitor of membrane lipid peroxidation (8). α-Tocopherol also scavenges peroxyl radicals, singlet oxygen (9), and superoxide anion radicals (10). The succinated form of natural *d*-α-tocopherol has been demonstrated to be the most effective antiproliferative agent in tumor cells *in vitro* (11).

Phenobarbital (PB) is a well-recognized hepatopromoter and inducer of cytochrome *P*-450s. It is an effective promoter of hepatic preneoplasms and tumor development, although the underlying mechanisms of this promotion are unclear (12,13). PB is used as a promoter in several models, including those developed by Ito and co-workers (12) in a study of multistage hepatocarcinogenesis as quantified by altered hepatic foci (13). Altered hepatic foci are preneoplastic lesions that develop during multistage hepatocarcinogenesis (14), and they reflect clonal development from single initiated hepatocytes (15,16).

Increased production of prostaglandins (PG) has been shown to influence carcinogenesis by stimulating tumor growth (17) or affecting tumor migration and the metastatic potential (18). Increased secretion of $PGE_2$ and $PGF_{2\alpha}$ into cell culture medium has been found in various non-small-cell lung carcinomas (19,20). E-type PGs modulate human keratinocyte proliferation *in vitro* and *in vivo* (21,22). The gen-

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eral response of tissues to chemical and mechanical injury is hyperplastic transformation, which has been found to be mediated by E-type PGs (22). Vitamin E inhibits lung $PGE_2$ production after urethan treatment in mice (23), and addition of vitamin E to macrophage cultures decreases $PGE_2$ production and improves T cell proliferation and interleukin-2 production (24).

Protein kinase C (PKC) is recognized to play an important role in carcinogenesis, and PKC expression is a potential marker for malignant diseases (25). Drugs that inhibit PKC activity are believed to inhibit tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate (26). Thus the findings that *in vitro* administration of vitamin E decreases the activity of PKC purified from brain and aorta smooth muscle cells argue for a role of this vitamin in the inhibition of tumor promotion (27,28). Moreover, macrophages from vitamin E-treated rats were also shown to have decreased PKC activities (29).

ROS are implicated in initiation and promotion stages of carcinogenesis, and α-tocopherol is an effective radical scavenger. In addition to its antioxidant role, α-tocopherol affects a number of parameters that are intimately involved in tumorigenesis (e.g., $PGE_2$ and PKC). The aim of this study was to investigate the effects of α-tocopherol succinate on tumor formation in a diethylnitrosamine (DEN) initiation-PB promotion model of hepatocarcinogenesis and the involvement of lipid peroxidation, $PGE_2$, and PKC in this model.

## Materials and Methods

### Animals and Diets

Pregnant Sprague-Dawley rats were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). Female pups were initiated at 24 hours of age by injection of DEN (15 mg/kg ip) in phosphate-buffered saline (pH 7.0). We chose female Sprague-Dawley rats, because in a previous study Sprague-Dawley rats were reported to be preferable to Fischer rats and females were preferable to males for this DEN initiation-PB promotion model of hepatocarcinogenesis (30). This model can produce a rapid induction of maximal yields of foci and tumors with minimal carcinogen treatment. Twenty-four weanling rats were randomly assigned to experimental diets. The experimental diets were nutritionally complete and provided 30% of energy as fat (Table 1). Diets were α-tocopherol deficient (0 ppm), adequate (100 ppm), or supplemented (5,000 ppm). The three groups of rats were provided ad libitum access to feed and tap water for 24 weeks.

### Plasma Preparation

After 24 weeks of feeding, the rats were fasted overnight and killed by an overdose of $CO_2$. Blood was drawn from the jugular vein for plasma $PGE_2$ assay and from the dorsal vein for plasma α-tocopherol determination. Nine parts of blood were added to one part of the anticoagulant (50 mM EDTA). For the endogenous $PGE_2$ assay, anticoagulant containing 0.7 mg/ml indomethacin was used. The blood was put into a centrifuge tube, and the tube was gently inverted. Plasma was obtained by centrifugation of blood at 1,500 *g* for five minutes. Plasma was removed after centrifugation and stored at −80°C for later analysis.

**Table 1.** Composition of Experimental Diets[a,b]

| Ingredient | α-Tocopherol Group | | |
|---|---|---|---|
| | Deficient | Adequate | Supplemented |
| Corn oil, α-tocopherol stripped | 15 | | |
| Corn oil | | 15 | 15 |
| Casein | 15 | 15 | 15 |
| Dextrose | 15 | 15 | 15 |
| Cornstarch | 45 | 45 | 45 |
| Cellulose | 4.95 | 4.95 | 4.45 |
| Vitamin mix, tocopherol devoid | 1 | | |
| AIN vitamin mix | | 1 | 1 |
| AIN mineral mix | 3.5 | 3.5 | 3.5 |
| Choline bitartrate | 0.2 | 0.2 | 0.2 |
| DL-Methionine | 0.3 | 0.3 | 0.3 |
| α-Tocopheryl succinate | | | 0.5 |
| Phenobarbital | 0.05 | 0.05 | 0.05 |

*a:* Values are g/100 g.
*b:* Ingredients were obtained from Teklad (Madison, WI), except for phenobarbital, which was obtained from Sigma Chemical (St. Louis, MO).

### Plasma and Liver α-Tocopherol Levels and Lipid Peroxidation Assays

Hepatic and plasma α-tocopherol concentrations were determined by means of a modification of the procedure of Catignani and Bieri (31). Fifty microliters of an internal standard (α-tocopheryl acetate in ethanol) and hepatic homogenate (100 µl taken from 0.1 g of liver-1 ml of 50 mM potassium phosphate buffer, pH 7.0) or 100 µl of plasma were mixed by vortexing for one minute. To extract the lipid, 200 µl of high-performance liquid chromatography (HPLC)-grade hexane were added, and the suspension was mixed for an additional one minute. Phases were separated by centrifugation at 2,000 rpm for two minutes, and the hexane layer was withdrawn and evaporated under nitrogen. The residue was redissolved in 50 µl of filtered HPLC-grade methanol by mixing, and 20 µl of the mixture were injected into an HPLC instrument. The HPLC instrumentation was purchased from Hitachi (Tokyo, Japan) and consisted of a model L-6200A intelligent pump, a model L-4200 UV-VIS detector, a model D-6000 interface, and an LC organizer. The column was 3.9 mm × 30 cm stainless steel packed with micro-Bondapak C-18. A 3 × 22 mm guard column (precolumn) packed with Bondapak C-18 was attached to the primary column. The detector wavelength was 290 nm, with a sensitivity of 0.01 absorbance unit full scale. The solvent was 100% HPLC-grade methanol, and the flow rate was 1.2 ml/min. Peak-to-area ratios of samples were con-

verted to micrograms of α-tocopherol by use of a standard curve prepared with samples containing a constant amount of α-tocopherol acetate combined with different amounts of α-tocopherol standard. Plasma total lipid was determined by the sulfophosphovanillin reaction, as described by Frings and Dunn (32).

Liver lipid peroxidation was measured by assaying thiobarbituric acid-reactive substances (TBARS) with use of a modification of the procedure described by Fraga and colleagues (33). Briefly, liver samples were homogenized in 50 mM potassium phosphate buffer (pH 7.4). To the liver homogenate were added 0.5 ml of 3% sodium dodecyl sulfate, 2 ml of 0.1 N HCl, 0.3 ml of 10% phosphotungstic acid, and 1 ml of 0.7% 2-thiobarbituric acid. The mixture was heated in boiling water for 30 minutes, and TBARS were extracted into 5 ml of 1-butanol. After centrifugation, the fluorescence of the butanol layer was measured at 515-nm excitation and 555-nm emission in a Hitachi F-4500 fluorescence spectrophotometer. The values were expressed in nanomoles per gram of liver. A malondialdehyde standard curve was also prepared using 1,1,3,3-tetramethoxypropane.

### Hepatic GSH Redox Status Assay

Frozen liver tissue was used to determine the GSH redox status. GSH and GSSG were determined by HPLC, as described by Reed and associates (34), with some modifications (35).

### Hepatic Antioxidant Enzyme Activity Assays

Hepatic cytosolic and microsomal fractions of rats were prepared by differential centrifugation (36). Hepatic cytosolic GSH peroxidase activity was determined spectrophotometrically with a coupled procedure in which $H_2O_2$ was used as the substrate (37). Hepatic cytosolic GSH reductase activity was measured as described by Bellomo and others (38). Hepatic glutathione *S*-transferase (GST) activity was determined by the method of Habig and associates (39). Samples and reference cuvettes were read for five minutes in a dual-beam spectrophotometer set at 340 nm. Activity was expressed as nanomoles of 1-chloro-2,4-dinitrobenzene conjugate formed per milligram of protein per minute. Protein content was determined by the method of Lowry and co-workers (40).

### Plasma $PGE_2$ Analysis

The plasma $PGE_2$ content was analyzed by radioimmunoassay (RIA). The $PGE_2$ $^{125}I$ RIA kit was obtained from New England Nuclear (Boston, MA). The RIA systems have the high-sensitivity characteristics.

### Protein Kinase C Activity Assay

Hepatic protein kinase C (PKC) activity was determined according to the method described by Chang and colleagues (41), and partial purification of the enzyme was performed. Hepatic supernatants of cytosolic and membranous fractions were applied to 0.5-ml DEAE-cellulose columns equilibrated in homogenization Buffer B minus Triton X-100. Columns were washed with 5 ml of equilibration buffer, and PKC activity was eluted with 2 ml of equilibration buffer containing 0.2 M KCl. The elution was used for PKC activity determination.

### Hepatic Altered Foci Analysis

The largest lobes of the rat liver were cut into 1-cm-thick slices, frozen on dry ice, and stored at −80°C. Frozen liver slices were further sliced into 10-μm serial sections for placental form of GST (P-GST)- and γ-glutamyl transpeptidase (γ-GT)-positive focus assays. P-GST-positive foci were visualized by immunohistochemical methods, as described by Hendrich and associates (42). Rabbit anti-P-GST antiserum was kindly provided by Dr. Hendrich (Iowa State University, Ames, IA). P-GST-positive foci were detected with a Vectastain ABC/peroxidase immunoassay kit (Vector Laboratories, Burlingame, CA). For color development, aminoethylcarbazole (AEC kit, Vector Laboratories) was used as the substrate for peroxidase.

γ-GT-positive focus assays were performed according to the method described by Rutenburg and others (43). Briefly, the 10-μm liver sections were air-dried, submersed in freshly prepared γ-glutamyl-4-methoxy-2-naphthylamine solution for 15 minutes at room temperature, washed in 0.85% saline solution for 2 minutes, and stabilized with 0.1 M $CuSO_4$ for 2 minutes, washed in deionized water, and air dried. Finally, glycerol gelatin was added and the slides were covered with coverslips. The sizes and numbers of γ-GT- and P-GST-positive hepatic foci were quantified under a microscope with Leica Q500MC software. The foci were recognized when their diameter was >0.25 mm.

### Statistical Analysis

All analyses were conducted in duplicate for each sample. Data were analyzed by using analysis of variance (SAS Institute, Cary, NC). Tukey's test was used to evaluate the significance of the difference between means; $p < 0.05$ was taken to be statistically significant.

## Results

The level of dietary α-tocopherol had no significant effect on food intake, body weight gain, or liver weight of the rats. Rats fed α-tocopherol-deficient diets had significantly greater spleen weight than those fed α-tocopherol-supplemented diets (0.63 ± 0.09 and 0.48 ± 0.09 g, respectively, $p < 0.05$), but there was no difference in spleen weight of rats fed α-tocopherol-deficient or -adequate diets. Rats fed α-tocopherol-deficient diets also had significantly greater liver weight as a percentage of body weight than rats fed α-tocopherol-adequate or -supplemented diets (3.5 ± 0.2%,

**Table 2.** Plasma and Liver α-Tocopherol Concentration, Liver Lipid Peroxidation, and GSH-to-GSSG and GSH-to-Total GSH Ratios of Rats Fed Different Levels of α-Tocopherol[a,b]

| α-Tocopherol Group | Plasma α-Tocopherol, μg/mg lipid | Liver α-Tocopherol, μg/g liver | Liver TBARS, nmol/g liver | Liver GSH-to-GSSG Ratio | Liver GSH-to-Total GSH Ratio |
|---|---|---|---|---|---|
| Deficient | 1.6 ± 2.1‡ | 9.7 ± 7.1† | 99.4 ± 37.1* | 21.9 ± 2.8 | 0.92 ± 0.01 |
| Adequate | 6.1 ± 2.0† | 27.3 ± 12.6† | 54.0 ± 7.3† | 25.2 ± 1.7 | 0.93 ± 0.01 |
| Supplemented | 12.5 ± 1.7* | 276.8 ± 139.2* | 41.3 ± 4.5† | 25.6 ± 4.9 | 0.93 ± 0.02 |

*a:* Values are means ± SD of 8 rats in each group. GSH, reduced glutathione; GSSG, oxidized glutathione; TBARS, thiobarbituric acid-reactive substances.
*b:* Groups not sharing a symbol (*,†,‡) are significantly different ($p < 0.05$).

**Table 3.** Hepatic GST, GSH Reductase, and GSH Peroxidase Activities of Rats Fed Different Levels of α-Tocopherol[a,b]

| α-Tocopherol Group | GST, nmol/mg protein/min | GSH Reductase, nmol/mg protein/min | GSH Peroxidase, nmol/mg protein/min |
|---|---|---|---|
| Deficient | 3,384 ± 616† | 58.0 ± 8.6† | 618 ± 114† |
| Adequate | 4,415 ± 732* | 74.9 ± 11.3* | 1,062 ± 165* |
| Supplemented | 4,391 ± 694* | 71.9 ± 15.5* | 913 ± 215* |

*a:* Values are means ± SD of 8 rats in each group. GST, glutathione *S*-transferase.
*b:* Groups not sharing a symbol (*,†) are significantly different ($p < 0.05$).

3.1 ± 0.3%, and 3.1 ± 0.3%, respectively, $p < 0.05$); however, there was no difference in liver weight as a percentage of body weight of rats fed α-tocopherol-adequate or -supplemented diets.

The plasma α-tocopherol concentrations of rats were significantly affected by the level of dietary α-tocopherol ($p < 0.05$). Rats fed α-tocopherol-supplemented diets had significantly greater hepatic α-tocopherol content than those fed α-tocopherol-adequate or -deficient diets ($p < 0.05$), and rats fed α-tocopherol-adequate diets had significantly greater plasma levels than those fed α-tocopherol-deficient diets ($p < 0.05$) (Table 2). Rats fed α-tocopherol-deficient diets had significantly greater liver lipid peroxidation (measured as TBARS) than those fed α-tocopherol-adequate or -supplemented diets ($p < 0.05$), but the liver lipid peroxidation of rats fed the α-tocopherol-adequate diet was not significantly different from that of rats fed the α-tocopherol-supplemented diet (Table 2). The liver GSH redox status was not affected by the dietary α-tocopherol level (Table 2).

Rats fed α-tocopherol-adequate and -supplemented diets had significantly greater hepatic GST, GSH reductase, and GSH peroxidase activities than those fed α-tocopherol-deficient diets ($p < 0.05$); however, those activities of rats fed α-tocopherol-adequate or -supplemented diets were not significantly different (Table 3). The dietary α-tocopherol content had no significant effect on plasma $PGE_2$ concentration or on the activities of hepatic cytosolic or particulate PKC (Table 4). Dietary α-tocopherol had no effect on the size or number of γ-GT and P-GST-positive hepatic foci (Table 5).

**Table 4.** Plasma $PGE_2$ Level and Hepatic Cytosolic and Particulate PKC Activities of Rats Fed Different Levels of α-Tocopherol[a]

| α-Tocopherol Group | Plasma $PGE_2$, ng/ml | PKC, pmol/mg protein/min | |
|---|---|---|---|
| | | Cytosolic | Particulate |
| Deficient | 4.17 ± 0.60 | 84.3 ± 56.0 | 639 ± 337 |
| Adequate | 3.86 ± 1.05 | 72.8 ± 43.1 | 615 ± 181 |
| Supplemented | 3.04 ± 0.61 | 103.3 ± 63.1 | 694 ± 281 |

*a:* Values are means ± SD of 8 rats in each group. PKC, protein kinase C; $PGE_2$, prostaglandin $E_2$.

## Discussion

The finding that spleen weight and liver weight as a percentage of body weight were significantly greater in α-tocopherol-deficient rats than in α-tocopherol-supplemented rats may imply that dietary α-tocopherol is involved in modulation of detoxification and immune activities of animals, as suggested by other studies (44,45).

The plasma and liver α-tocopherol status was affected by the dietary α-tocopherol level (Table 2), consistent with the results of our previous study (44). Liver lipid peroxidation was significantly greater in rats fed α-tocopherol-deficient diets than in those fed α-tocopherol-adequate or -supplemented diets (Table 2). α-Tocopherol is a well-known lipid-soluble antioxidant, and it can prevent or inhibit lipid peroxidation (8). In the present study, the α-tocopherol-adequate diet inhibited lipid peroxidation significantly compared with the deficient diets, and supplementation with additional α-tocopherol succinate offered a small but insignificant additional protective effect. The dietary α-tocopherol level did not significantly affect the ratio of reduced GSH to GSSG in the liver (Table 2). This result is consistent with that of our previous study in which red blood cells were more sensitive than the liver to dietary vitamin E manipulation, as determined on the basis of the GSH status (44).

Vegetables and fruits have been suggested to possess anticarcinogenic properties, because they contain a large number of inhibitors of carcinogenesis, namely, phenols, indoles, aromatic isothiocyanates, ascorbic acid, α-tocopherol, and carotenes (46,47). The anticarcinogenic mecha-

**Table 5.** Areas and Numbers of Hepatic γ-GT- and P-GST-Positive Foci of Rats Fed Different Levels of α-Tocopherol[a]

| Foci Area and Number | α-Tocopherol Group | | |
|---|---|---|---|
| | Deficient | Adequate | Supplemented |
| Area occupied by γ-GT-positive foci, % | 1.7 ± 0.7 | 1.1 ± 0.5 | 2.1 ± 1.4 |
| γ-GT-positive foci | | | |
| no./cm$^2$ | 9.8 ± 4.2 | 11.8 ± 5.9 | 15.4 ± 6.8 |
| no./cm$^3$ | 132 ± 70 | 187 ± 93 | 223 ± 86 |
| Area occupied by P-GST-positive foci, % | 2.5 ± 0.9 | 1.7 ± 0.6 | 2.9 ± 1.6 |
| P-GST-positive foci | | | |
| no./cm$^2$ | 15.5 ± 6.1 | 17.4 ± 8.0 | 17.7 ± 5.7 |
| no./cm$^3$ | 226 ± 102 | 274 ± 143 | 253 ± 107 |

*a:* Values are means ± SD of 8 rats in each group. γ-GT, γ-glutamyl transpeptidase; P-GST, placental GST.

nisms of these compounds are poorly understood, although enhancement of carcinogen detoxification may be involved (46). GST catalyzes the binding of a variety of electrophiles to the sulfhydryl group of GSH. Because the ultimate forms of carcinogens are electrophiles, GST plays an important role in anticarcinogenesis through its detoxification function (48). Oxidative damage is well recognized to be involved in the multistage process of carcinogenesis (1). GSH peroxidases catalyze the reduction of organic hydroperoxides and $H_2O_2$ (5), whereas GSH reductase regenerates reduced GSH from GSSG, an important step in the detoxification process. The hepatic GST, GSH reductase, and GSH peroxidase activities were significantly affected by the dietary α-tocopherol level in our study: rats fed α-tocopherol-adequate and -supplemented diets had significantly greater activities than those fed α-tocopherol-deficient diets ($p < 0.05$) (Table 3). In our previous study, we found that rats fed α-tocopherol-adequate and -supplemented diets had significantly greater hepatic GST activity than those fed no α-tocopherol (49). These results indicate that the inhibitory effect of α-tocopherol on carcinogenesis may be mediated through its influence on the activities of antioxidant and detoxification enzymes.

Acetylsalicylic acid, an inhibitor of cyclooxygenase, has been shown to significantly decrease the number of hepatocellular carcinomas in the presence or absence of PB. This finding suggests the involvement of arachidonic acid metabolites in the evolution of preneoplastic foci into nodules and hepatocellular carcinomas in the rat liver, with or without PB exposure (50). In addition to its role in carcinogenesis, arachidonic acid metabolites (e.g., $PGE_2$) are also involved in immunosuppression. A variety of tumor cells can greatly stimulate production of $PGE_2$ by macrophages; these PGs in turn inhibit the production of lymphokines, which are essential for normal function of lymphocytes. This indicates that PGs may be responsible for the escape of tumor cells from normal immune surveillance (51). Dietary vitamin E affects the immune response, and it has been shown to enhance mitogen-stimulated lymphocyte proliferation and delayed-type skin hypersensitivity in aging mice. This is considered to be closely associated with decreased $PGE_2$ synthesis (52). In the present study, the plasma $PGE_2$ level was not significantly affected by the dietary α-tocopherol level, although we did find a trend of decreasing plasma $PGE_2$ levels with α-tocopherol supplementation (Table 4). Dietary α-tocopherol did not significantly affect the activity of hepatic cytosolic or membrane-bound PKC (Table 4), and this finding conflicts with the results of previous studies (27–29).

Vitamin E has been shown to inhibit induction of P-GST-positive foci in the liver of rats initiated with DEN (53). Fischer 344 rats were treated with 200 mg DEN/kg body wt, fed a basal diet for two weeks, and then fed a diet containing 1.5% *dl*-α-tocopherol acetate for six weeks. All animals were subjected to partial hepatectomy three weeks after DEN administration. In another study (54), skin tumors were produced by a two-stage initiation-promotion treatment regimen in female Skh-1 hairless mice, and vitamin E was found to decrease the number of chemically induced skin tumors at Week 27. Also, in male Fischer rats, vitamin E (1.5%) was found to inhibit the initiation of hepatocarcinogenesis by the food carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (100 mg/kg ig) after an 11-week study (55). However, in some experiments, no anticarcinogenic effect or even an enhancing effect on liver tumorigenesis and skin tumor promotion was found (26,56). In this study, dietary α-tocopherol had no effect on the size or number of γ-GT- and P-GST-positive hepatic foci, although it showed significant effects on hepatic α-tocopherol status, antioxidant enzyme activities, and lipid peroxidation (Table 5).

In conclusion, the level of dietary α-tocopherol had no effect on the formation of γ-GT- and P-GST-positive hepatic foci in this DEN initiation-PB promotion model of hepatocarcinogenesis. α-Tocopherol significantly affected the hepatic α-tocopherol status, antioxidant enzyme activities, and lipid peroxidation, suggesting that ROS may not be the underlying mechanism in this model of hepatocarcinogenesis.

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