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

Chong-Kuei Lii, Chien-Wei Chen, Jer-Yuh Liu, Yuh-Jane Ko, and Haw-Wen Chen

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 E2 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 H2O2 to H2O 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 succinylated form of natural 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 PGE2 and PGF2α 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-
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 PGE2 production after urethan treatment in mice (23), and addition of vitamin E to macrophage cultures decreases PGE2 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., PGE2 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, PGE2, 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 CO2. Blood was drawn from the jugular vein for plasma PGE2 assay and from the dorsal vein for plasma α-tocopherol determination. Nine parts of

| Table 1. Composition of Experimental Diets<sup>a,b</sup> |
|---------------------------------|---------------|---------------|---------------|
| **Ingredient**                  | **Deficient** | **Adequate**  | **Supplemented** |
| Corn oil, α-tocopherol stripped | 15            | 15            | 15            |
| Corn oil                        | 15            | 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             | 1             | 1             |
| AIN vitamin mix                 | 3.5           | 3.5           | 3.5           |
| AIN mineral mix                 | 0.2           | 0.2           | 0.2           |
| Choline bitartrate              | 0.3           | 0.3           | 0.3           |
| DL-Methionine                   | 0.3           | 0.3           | 0.3           |
| α-Tocopheryl succinate          | 0.5           | 0.5           | 0.5           |
| Phenobarbital                   | 0.05          | 0.05          | 0.05          |

<sup>a</sup> Values are g/100 g.

<sup>b</sup> Ingredients were obtained from Teklad (Madison, WI), except for phenobarbital, which was obtained from Sigma Chemical (St. Louis, MO).

Blood was added to one part of the anticoagulant (50 mM EDTA). For the endogenous PGE2 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.

#### 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 was 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 thio- 

barbituric acid-reactive substances (TBARS) with use of a 

modification of the procedure described by Fraga and co-

leagues (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 sul-

fate, 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 modifica-

tions (35).

Hepatic Antioxidant Enzyme Activity Assays

Hepatic cytosolic and microsomal fractions of rats were 

prepared by differential centrifugation (36). Hepatic cyto-

solic GSH peroxidase activity was determined spectropho-

tometrically with a coupled procedure in which H2O2 

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 asso-

ciates (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 PGE2 Analysis

The plasma PGE2 content was analyzed by radioimmu-

nunassay (RIA). The PGE2 125I 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 equili-

brated 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 pla-

cental 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 anti-

serum was kindly provided by Dr. Hendrich (Iowa State 

University, Ames, IA). P-GST-positive foci were detected with 

a Vectastain ABC/peroxidase immunohistoassay kit (Vector 

Laboratories, Burlingame, CA). For color development, ami-

noethylcarbazole (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 CuSO4 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 quantitated 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-supple-

mented 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$^{ab}$

<table>
<thead>
<tr>
<th>α-Tocopherol Group</th>
<th>Plasma α-Tocopherol, µg/mg lipid</th>
<th>Liver α-Tocopherol, µg/g liver</th>
<th>Liver TBARS, nmol/g liver</th>
<th>Liver GSH-to-GSSG Ratio</th>
<th>Liver GSH-to-Total GSH Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td>1.6 ± 2.1$^4$</td>
<td>9.7 ± 7.1$^1$</td>
<td>99.4 ± 37.1$^*$</td>
<td>21.9 ± 2.8</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>Adequate</td>
<td>6.1 ± 2.0$^1$</td>
<td>27.3 ± 12.6$^1$</td>
<td>34.0 ± 7.3$^1$</td>
<td>25.2 ± 1.7</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>Supplemented</td>
<td>12.5 ± 1.7$^*$</td>
<td>276.8 ± 139.2$^*$</td>
<td>41.3 ± 4.5$^*$</td>
<td>25.6 ± 4.9</td>
<td>0.93 ± 0.02</td>
</tr>
</tbody>
</table>

*a: Values are means ± SD of 8 rats in each group. GSH, reduced glutathione; GSSG, oxidized glutathione; TBARS, thiobarbituric acid-reactive substances.

Table 3. Hepatic GST, GSH Reductase, and GSH Peroxidase Activities of Rats Fed Different Levels of α-Tocopherol$^{ab}$

<table>
<thead>
<tr>
<th>α-Tocopherol Group</th>
<th>GST, nmol/mg protein/min</th>
<th>GSH Reductase, nmol/mg protein/min</th>
<th>GSH Peroxidase, nmol/mg protein/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td>3.384 ± 616$^1$</td>
<td>58.0 ± 8.6$^1$</td>
<td>618 ± 114$^1$</td>
</tr>
<tr>
<td>Adequate</td>
<td>4.415 ± 732$^*$</td>
<td>74.9 ± 11.3$^*$</td>
<td>1.062 ± 165$^*$</td>
</tr>
<tr>
<td>Supplemented</td>
<td>4.391 ± 694$^*$</td>
<td>71.9 ± 15.5$^*$</td>
<td>913 ± 215$^*$</td>
</tr>
</tbody>
</table>

*a: Values are means ± SD of 8 rats in each group. GST, glutathione S-transferase.

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-supplemented or -deficient diets ($p < 0.05$), and rats fed α-tocopherol-supplemented 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-supplemented or -deficient diets ($p < 0.05$), but the liver lipid peroxidation of rats fed the α-tocopherol-deficient 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-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-supplemented or -deficient 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).

Talk 4. Plasma PGE$_2$ Level and Hepatic Cytosolic and Particulate PKC Activities of Rats Fed Different Levels of α-Tocopherol

<table>
<thead>
<tr>
<th>α-Tocopherol Group</th>
<th>Plasma PGE$_2$, ng/ml</th>
<th>Cytosolic PKC, pmol/mg protein/min</th>
<th>Particulate PKC, pmol/mg protein/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td>4.17 ± 0.60</td>
<td>84.3 ± 56.0</td>
<td>639 ± 337</td>
</tr>
<tr>
<td>Adequate</td>
<td>3.86 ± 1.05</td>
<td>12.8 ± 43.1</td>
<td>615 ± 181</td>
</tr>
<tr>
<td>Supplemented</td>
<td>3.04 ± 0.61</td>
<td>103.3 ± 63.1</td>
<td>694 ± 281</td>
</tr>
</tbody>
</table>

*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-supplemented or -adequate diets (Table 2). α-Tocopherol is a well-known liposoluble antioxidant, and it can prevent or inhibit lipid peroxidation (8). In the present study, the α-tocopherol-deficient 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 carotenoids (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

<table>
<thead>
<tr>
<th>Foci Area and Number</th>
<th>α-Tocopherol Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient</td>
</tr>
<tr>
<td>Area occupied by γ-GT-positive foci, %</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>no. cm²</td>
<td>9.8 ± 4.2</td>
</tr>
<tr>
<td>no. cm³</td>
<td>132 ± 70</td>
</tr>
<tr>
<td>Area occupied by P-GST-positive foci, %</td>
<td>2.5 ± 0.9</td>
</tr>
<tr>
<td>no. cm²</td>
<td>15.5 ± 6.1</td>
</tr>
<tr>
<td>no. cm³</td>
<td>226 ± 102</td>
</tr>
</tbody>
</table>

α: 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 H₂O₂ (5), whereas GSH reductase regenerates reduced GSH from GSSSH, 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., PGE₂) are also involved in immunosuppression. A variety of tumor cells can greatly stimulate production of PGE₂ 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 PGE₂ synthesis (52). In the present study, the plasma PGE₂ level was not significantly affected by the dietary α-tocopherol level, although we did find a trend of decreasing plasma PGE₂ 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) 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.

Acknowledgments and Notes

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