行政院國家科學委員會專題研究計畫 成果報告

炸油或藥物投與對維生素E代謝途徑之探討
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 96-2320-B-041-008-
執 行 期 間 ：96年08月01日至97年07月31日
執 行 單 位 ：嘉南藥理科技大學保健營養系

計 畫 主 持 人：蕭慧美

計畫參與人員：碩士班研究生-兼任助理人員：黃玟綺
碩士班研究生-兼任助理人員：陳婉宣

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 97年07月14日

Vitamin-E Supplementation Restored Hepatic Vitamin-E Levels but not Protein Levels of Antioxidant Enzymes Decreased by Administration of Pregnenolone-16α-Carbonitrile in Rats

HUEY-MEI SHAW[1*], WAN-HSUAN CHEN[2]

[1]*Department of Health and Nutrition, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, mei@mail.chna.edu.tw*

[2]*Institute of Health and Nutrition Science, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan*

Summary Pregnenolone-16α-carbonitrile (PCN) is a synthetic steroid and an inducer of the cytochrome P450 3A gene in rats. The aim of this study was to evaluate the effects of PCN administration on vitamin-E status and antioxidant enzyme protein levels in rats fed a vitamin-E supplemented diet. Two groups of Wistar rats were fed for three weeks with a basal diet (containing 50 ppm of α-tocopherol) or the same diet containing tenfold more α-tocopherol. In the last three days, each group was divided into two subgroups which were given a single intraperitoneal injection of PCN at 75 mg/kg/day (P50 and P500 groups) or DMSO (C50 and C500 groups). PCN significantly reduced α-tocopherol contents in the liver and plasma. Tenfold supplementation of α-tocopherol (P500 group) could return liver α-tocopherol levels to that of the C50 group. The TBARS concentration was significantly elevated by PCN administration in the liver and lung by Two-way ANOVA analysis. PCN showed a significant reduction in the protein levels of catalase and glutathione peroxidase

(GPx). Dietary vitamin-E supplementation altered the protein levels of GPx and superoxide dismutase, but not catalase. Vitamin-E supplementation protected against PCN-induced lipid peroxidation and compromized vitamin-E status by CYP3A induction and antioxidant enzyme reduction. The mechanism of this protection appears to be due to scavenging the reactive metabolite by vitamin-E rather than increasing antioxidant enzyme levels.

Key Words α-tocopherol, TBARS, antioxidant enzyme, cytochrome P450, rat

Cytochrome P450s (CYPs) are part of the monooxygenases superfamily, which are responsible for xenobiotic metabolism. The reaction steps of CYPs involve single-electron transfers, which can give rise to byproducts such as superoxide and hydrogen peroxide (1-3). Excessive production of these reactive oxygen species can damage lipids, proteins and DNA.

Vitamin-E is an important fat-soluble antioxidant in the body. It works with antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase to protect cells from attack by reactive oxygen species.

Vitamin-E has been reported to attenuate the decrease of CYP1A by polymicrobial sepsis (4). Several studies also showed that α-tocopherol can induce CYP proteins; the activities of CYP1A1, CYP2C, and CYP2B increased in rat liver microsomes after α-tocopherol injection (5) and liver CYP3A and CYP2B proteins were induced in rats fed a α-tocopherol supplementation diet (6). A high dose (200 mg) of α-tocopherol in the diet for three months induced CYP3a11 in mice compared with mice fed a α-tocopherol-deficient diet (7). These results reveal that vitamin-E is related to regulation of CYP proteins.

Tocopherols are metabolized by ω-hydroxylation followed by β-oxidation; some CYPs are thought to exhibit ω-hydroxylase activity. CYP3A was first reported to be involved in vitamin-E metabolism by determining changes in carboxyethyl-hydroxychroman CEHC metabolites in HepG2 cells when a CYP inhibitor, ketoconazole (KCZ), or an inducer, rifampicin, was added to the medium (8,9).

CYP3A in the liver is responsible for >50% of the metabolism of drugs (10). CYP3A4 protein was induced in alcoholic liver disease and non-alcoholic fatty liver in which lipid peroxidation-derived protein adducts increased (11). The level of

α-glutathione-S-transferase protein increased in CYP3A4 expression in the HepG2 cell (12). These studies suggest that CYP3A induction is accompanied by increased oxidative stress, which contributes to liver injury. Vitamin-E and antioxidant enzymes could be altered to protect liver cells, but little is known about the status of vitamin-E and antioxidant enzymes when CYP3A protein is induced in the liver.

Pregnenolone-16α-carbonitrite (PCN) is a synthetic steroid. A microsomal enzyme inducer has been shown to activate the pregnane X receptor (PXR), which has a critical role in the induction of CYP3A genes in response to PCN (13,14). We previously reported that oxidative stress increased after PCN administration. In the current study, the status of vitamin-E in tissues and protein levels of hepatic antioxidant enzymes after PCN administration when supplemented with vitamin-E in the diet were evaluated in rats.

.

Materials and Methods

Animals and diets The care and handling of animals conformed to accepted guidelines (15). Male Wistar rats (age, 3 weeks; BioLASCO Company, Taipei, Taiwan) were housed individually in stainless-steel wire cages in a room maintained at 23 ± 2°C with a controlled 12-h light/dark cycle. They had free access to water and food. Body weights were recorded every week. Rats were fed a purified basal diet according to AIN-76 diet before individual treatments.

Rats (body weight, about 93 ± 7 g) were randomly assigned to two groups which were fed basal diet alone (containing 50 ppm of α-tocopherol) or with an added high level of vitamin-E (500 ppm of α-tocopherol) for three weeks (body weight, 252 ± 22 g). In the last three days, each group was divided into two subgroups, which were given an injection (i.p.) of 75 mg/kg of 5-pregnen-3b-01-20-one-16a-carbonitrite (PCN; groups P50 and P500) or dimethylsulfoxide (DMSO groups D50 and D500) per day for three days (16).

Sampling and preparation of tissue At the end of the feeding period, food was withheld overnight and rats killed by carbon dioxide asphyxiation in the morning. Blood was collected from the abdominal vena cava into a heparinized tube and centrifuged at 1,000 × g for 10 min at room temperature, and the plasma stored at –80°C. A small piece of tissue was homogenized in ice-cold 0.01 mol/L phosphate buffer (containing 0.155 mol/L KCl, pH 7.4) using a Potter–Elvehjem-type homogenizer with a Teflon pestle. Liver homogenate was initially centrifuged at 12,000 × g at 4°C for 20 min. Supernatant was centrifuged again using an ultracentrifuge (Beckman) at 100,000 × g at 4°C for 1 h. The microsome pellet was resuspended in 0.05 M potassium phosphate buffer (containing 1 mM EDTA, pH 7.6) and the cytosolic supernatants stored at –70°C until analysis.

Measurement of concentrations of α-tocopherol and thiobarbituric acid-reactive substances (*TBARS)* The concentration of α-tocopherol in plasma and tissues was analyzed by HPLC as previously described (17). TBARS concentrations in the tissue homogenate were determined according to the method of Oteiza et al. (18).

Western blotting Primary antibodies against α-tocopherol transfer protein (α-TTP) were prepared as previously described (19) except they were raised in rabbits instead of Balb/c mice. Primary antibodies against antioxidant enzymes were purchased from Abcam (UK). Primary antibodies against CYP3A1 and 3A2 were purchased from Chemicon (USA). HRP-goat anti-rabbit IgG (H + L) conjugate was the secondary antibody (Zymed Company). Aliquots of the liver cytosolic fraction or microsome suspension solution containing 10 μg of protein were separated by SDS-polyacrylamide electrophoresis, and transferred to a polyvinylidene fluoride (PVDF) membrane, which was incubated overnight at 4°C with blocking buffer (0.25% gelatin, 0.15 M NaCl, 5 mM EDTA, 0.05% Tween 20, 50 mM Tris, pH 8.0). Proteins on the membrane were immunostained for 1 h at room temperature using antibodies against α-TTP, CYP3A, SOD, GPx or catalase. After three washes with washing buffer (phosphate-buffered solution containing 0.05% Tween 20, pH 7.0), membranes were incubated with secondary antibody at room temperature for 1 h, then washed three times, and the signal visualized by reaction with ECL-plus substrate (Amersham, UK) for 20–40 sec.

Statistical analyses Data are expressed as mean ± SD. The significance of differences between the four groups was analyzed by one-way ANOVA and Duncan's multiple range test using the General Linear Model of the SAS Package (SAS Institute, Cary, NC, USA). Two-way ANOVA was used to confirm the effects of dietary vitamin-E and PCN injection and their interaction. $P<0.05$ was considered statistically

significant.

Results

Animal growth and organ weights

Rats were fed a basal diet or high α-tocopherol diet (50 or 500 ppm; "50" and "500" groups, respectively) for three weeks and injected on the last three days with vehicle (C50 and C500 groups) or CYP3A inducer PCN (P50 and P500 groups).

Before injection, four groups of rats had similar body weight, food intake (D50, P50, D500, P500; 16.6 ± 1.2, 17.0 ± 1.4, 17.3 ± 1.8, 17.2 ± 1.0 g/day, respectively) and feed efficiency (52.8 ± 2.6, 51.3 ± 3.7, 51.7 ± 4.2, 52.1 ± 4.3%, respectively). PCN injection for three days significantly increased liver weight (Table 1). The weights of kidneys, lung, heart and testes of rats were not significantly different among the four groups. Liver CYP3A1 protein and CYP3A2 protein were significantly induced by PCN injection (P50 and P500), but the addition of vitamin-E appeared to have no effect on CYP3A proteins (Figure 1). In the two control groups (C50 and C500), CYP3A2 protein level was more obvious than CYP3A1 protein level. This suggests that CYP3A1 is a type of inducible form, but CYP3A2 is a constitutive form in the liver; this is consistent with other studies (20, 21).

Levels of liver α-tocopherol, α-TTP and TBARS

Levels of α-tocopherol in the plasma and liver in the two PCN-treated groups (P50, P500) were significantly reduced as determined by two-way ANOVA. α-Tocopherol levels in the kidney, lung, heart and testis were significantly higher in vitamin E-supplemented groups (C500 and P500) than those in normal vitamin-E groups (C50 and P50). Two-way ANOVA also showed that dietary vitamin-E significantly elevated vitamin-E concentration in these tissues (Table 2).

α-Tocopherol transfer protein was measured in this study because it is thought to

be important in regulating plasma vitamin-E. There was no significant difference in protein levels of α-TTP among the four groups (Figure 2).

Levels of TBARS of various tissues are shown in Table 3. PCN injection caused a significant increase in liver TBARS levels. There was no significant difference in TBARS levels in the kidney, lung, heart and testis among the four groups. PCN injection significantly decreased TBARS concentration in the lung. PCN and dietary vitamin-E showed an interaction on kidney TBARS levels. Dietary vitamin-E significantly reduced TBARS concentration in the heart and testis; this result was consistent with their vitamin-E levels.

Protein of SOD, GPx and catalase

The proteins of antioxidant enzymes were analyzed by Western blotting. SOD proteins were not significantly different among the four groups. Dietary vitamin-E significantly reduced SOD protein levels (Figure 3). Protein levels of GPx were significantly higher in the C50 group and significantly lower in the P500 group. Two-way ANOVA showed that dietary vitamin-E and PCN significantly reduced GPx protein levels (Figure 4).

Catalase showed different results from the other two enzymes. Protein levels of catalase were significantly higher in the P50 and P500 groups than in the C500 group, but were not significantly different compared with the C50 group. PCN injection produced a significant increase in catalase protein (Figure 5).

Discussion

PCN is a potent PXR activator and a well-known CYP3A inducer in the rodent (10). It also slightly induces expression of mRNAs for CYP2B2, CYP2C6, and CYP2C11 in the rat liver (22). This drug increased total P450 content (23), caused proliferation of smooth endothelial reticulum (24) and consequently increased liver weight and relative liver weight (23), which was also observed in our study. PCN injection greatly increased TBARS levels in the liver, suggesting that lipid peroxidation could be due to the induction of CYPs. Lipid peroxidation caused by PCN has not been investigated directly, but CYP3A-expressing microsomes are reported to have a higher rate of superoxide production than CYP1A1-expressing microsomes (25). Johnson et al. (16) reported that PCN injection increases the rate of excretion of biliary glutathione-derived sulfhydryl groups in rats. These data suggest that oxidative stress is increased by PCN due to induction of CYP. Except the liver, the TBARS levels of other organs measured showed no significant difference among the four groups. Two-way ANOVA indicated that PCN influenced the levels of TBARS in the liver and lung, but dietary vitamin-E influenced TBARS levels in the heart and testis. This result seems reasonable because the liver is the major target organ of PCN, and the kidney and lung were also reported to show CYP3A protein expression after PCN treatment in rats (26,27).

PCN treatment induced oxidative stress and reduced vitamin-E levels in the liver and plasma to 60% and 37%, respectively, simultaneously. When rats were given tenfold levels of vitamin-E in their diet, vitamin-E levels in plasma and the liver could return to the same levels as those in untreated rats. Higher levels of liver TBARS in PCN treatment were also reduced with tenfold vitamin-E supplementation. Two-way ANOVA showed that vitamin-E levels in the kidney, lung, heart and testis were not

changed by PCN, but by dietary vitamin-E. These results were in agreement with TBARS results, and suggest that the liver was the major organ in which CYP was highly induced by PCN. Vitamin-E levels of extrahepatic tissues were almost determined using lipoprotein vitamin-E from plasma. Levels of α-tocopherol in plasma and the liver were reduced after PCN injection, but vitamin-E levels in the kidney, lung, heart and testis were unchanged. Tenfold supplementation of vitamin-E in the diet increased vitamin-E levels to about 1.4–2 fold of that in extrahepatic tissues of normal vitamin-E groups. The half-lives for α-tocopherol were 7.6, 11.2, 13.3, and 33.3 days in the lung, kidney, heart and testis, respectively (28). The period of PCN injection was only three days shorter than the half-lives for α-tocopherol in tissues. This may partially explain why α-tocopherol levels in these tissues were unchanged by PCN, even plasma vitamin-E concentration was lower.

α-TTP, a cytosolic protein predominantly expressed in the mammalian liver (29,30), showed highly specific affinity for α-tocopherol (31). Reduction in plasma α-tocopherol levels could be due to the lower expression of α-TTP because α-TTP is thought to be important in the homeostasis of plasma α-tocopherol (32-36). Many studies have suggested that α-TTP is downregulated by oxidative stress. α-TTP mRNA levels were reduced and lipid peroxidation increased when rats were exposed to hyperoxia for 48 h (37). α-TTP mRNA and protein levels were also reduced in rat hepatoma (38) and galactosamine-induced liver injury (39). In our study, lipid peroxidation increased and plasma α-tocopherol decreased, but the protein levels of α-TTP did not decrease. This suggests that the regulation of α-TTP is PXR-independent, and is not relevant to CYP3A induction. The reduced α-tocopherol in plasma is due to the lower levels of α-tocopherol in the liver, not α-TTP levels.

CYP3A was reported to be involved in vitamin-E metabolism in primary hepatocyte cultures and HepG2 cells (8,40). Liver vitamin-E levels increased when rats were given the CYP3A inhibitor KCZ, (41). Reduction in liver α-tocopherol with PCN treatment can also result from enhanced degradation of vitamin-E because PCN is an inducer of CYP3A. Urine α-CEHC, a metabolite of α-tocopherol (8, 42,43) was measured in our previous study and did not increase after PCN administration (in press). This means that CYP3A induction does not enhance the degradation of α-tocopherol to α-CEHC, and this may not be the major reason for falling α-tocopherol levels in the liver with PCN treatment.

Antioxidant enzymes of SOD, GPx and catalase provide a defense system against reactive species. Changes in enzyme activity show their adaptation to oxidative stress. GPx and catalase, scavengers of hydrogen peroxide, were altered by PCN according to two-way ANOVA. Dietary vitamin-E also influenced GPx. The reduction of GPx protein in the P500 group showed the sparing effect of vitamin-E on GPx. Vitamin-E and GPx have at least partially interchangeable functions because vitamin-E supplementation decreases GPx activity in the rabbit aorta (44), rat brain (45) and red blood cell (RBC) without affecting hemolysis (46). This suggests that high levels of vitamin-E in tissues protects them from lipid peroxidation and spares the antioxidant enzymes; this could explain our observations. “Oxygen-responsive elements” is a specific sequence that is responsive to oxygen tension, and was found to be located in the 5’ flanking region of the human GPx gene (47). GPx could be indirectly regulated by vitamin-E or other oxidative factors.

Catalase protein decreased after PCN injection and did not reverse with tenfold supplemention of vitamin-E in the diet. Some studies have shown catalase activity (48) and its mRNA levels (49) in the liver were reduced in the hepatoma cell. Recently,

catalase gene expression was found to be downregulated in acute liver injury by carbon tetrachloride, which caused lipid peroxidation (50). It seems that catalase was downregulated due to liver injury. Our present study showed similar results, i.e., higher lipid peroxidation, but lower levels of catalase protein. Vitamin-E supplementation can restore vitamin-E levels in the liver, but the catalase protein levels remained low, indicating that catalase protein synthesis is not directly related to oxidative stress, and assumed to be relevant to PCN treatment. The relationship between CYP and enzymes has been reported by Mari and Cederbaum (12), catalase protein and mRNA expression was shown to increase in CYP2E1 overexpression in HepG2 cells, but was not induced in CYP3A4 expression cells. Activation was associated with decreased catalase activity in transgenic mice with human PXR (51). These data show that the regulation of catalase after PCN administration could be via a PXR-mediated pathway rather than mediated by oxidative stress.

PCN produced a significant reduction in the protein levels of catalase and GPx. Dietary vitamin-E altered the protein levels of GPx and SOD, but not catalase. In summary, vitamin-E supplementation protected against PCN-induced lipid peroxidation and compromized vitamin-E status by CYP3A induction and antioxidant enzyme reduction. The mechanism of this protection appears to be due to scavenging the reactive metabolite by vitamin-E, but not increasing antioxidant enzyme levels.

Acknowledgments

The Center of Biotechnology Research, Department of Pharmacy and Science are gratefully acknowledged for providing ultracentrifuge equipment. This work was financially supported by grants from the National Science Council (grant number NSC 96-2320-B-041-008), Taipei, Taiwan.

REFERENCES

1) Porter TD and Coon MJ. 1991. Cytochrome P450: Multiplicity of isoforms, substrates and regulatory mechanisms. *J Biol Chem* **266**: 13469-13472.

2) Halkier BA. 1996. Catalytic activities and structure/function relationships of cytochrome P450 enzymes. *Phytochemistry* **43**: 1-21.

3) White RE and Coon MJ. 1980. Oxygen activation by cytochrome P450. *Ann Rev Biochem* **49**: 315-356.

4) Kim JY, Lee SM. 2006. Vitamin C and E protect hepatic cytochrome P450 dysfunction induced by polymicrobial sepsis. *Eur J Pharmacol* **534**: 202-209.

5) Sidorova YA, Ivanova EV, Grishanova A Y, Lyakhovich VV. 2003. Dose-dependent effect of α-tocopherol on activity of xenobiotic metabolizing enzymes in rat liver. *Bull Exp Biol Med* **136**: 45-48.

6) Mustacich DJ, Leonard SW, Devereaux MW, Sokol RJ, Traber MG. 2006. α-Tocopherol regulation of hepatic cytochrome P450s and ABC transporters in rats. *Free Radical Biol Med* **41**: 1069-1078.

7) Kluth D, Landes N, Pfluger P, Muller-Schmehl K, Weiss K, Bumke-Vogt C, Ristow M, Brigelius-Flohe R. 2005. Modulation of Cyp3a11 mRNA expression by α-tocopherol but not γ-tocotrienol in mice. *Free Rad Biol Med* **38**: 507-514.

8) Birringer M, Drogan D, Brigelius-Flohe R. 2001. Tocopherols are metabolized in HepG2 cells by side chain ω-oxidation and consecutive β-oxidation. *Free Radic Biol Med* **31**: 226-232.

9) Sontag TJ, Parker RS. 2002. Cytochrome P-450 ω-hydroxylase pathway of tocopherol catabolism: novel mechanism of regulation of vitamin E status. *J Biol Chem* **277**: 25290-25296.

10) Kliewer SA, Goodwin B, Willson TM. 2002. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev* **23**: 687-702.

11) Niemela O, Parkkila S, Juvonen RO, Viitala K, Gelboin HV, Pasanen M. 2000. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. *J Hepatology* **33**: 893-901.

12) Mari M, Cederbaum AI. 2001. Induction of catalase, alpha, and microsomal glutathione S-transferase in CYP2E1 overexpression HepG2 cells and protection against short-term oxidative stress. *Hepatology* **33**: 652-661.

13) Lehmann Jm, McKee DD, Watson MA, Willson TM, Moore JT, Kliewer SA. 1998. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Investig* **102**: 1016-1023.

14) Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, Perlmann T, Lehmann JM. 1998. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92**:73-82.

15) National Research Council. Guide for the Care and Use of Laboratory Animals.1985; Publication no. 85-23 (rev.) National Institute of Health, Bethesda, MD.

16) Johnson DR, Habeebu SSM, Klaassen CD. 2002. Increase in bile flow and biliary excretion of glutathione-derived sulfhydryls in rats by drug-metabolizing enzyme inducers is mediated by multidrug resistance protein 2. *Toxicol Sci* **66**: 16-26.

17) Huang CJ, Shaw HM. 1994. Tissue vitamin E status is compromised by dietary protein insufficiency in young growing rats. *J Nutr* **124**: 571-579.

18) Oteiza PI, Olin KL, Fraga CG and Keen CL. 1995. Zinc deficiency causes oxidative damage to proteins, lipids and DNA in rat testes. *J Nutr* **125**: 823-839.

19) Shaw HM, Huang CJ. 1998. Liver α-tocopherol transfer protein and its mRNA are differentially altered by vitamin E deficiency and protein insufficiency in rats. *J Nutr* **128**: 2348-2354.

20) Schuetz EG, Wrighton SA, Barwick JL, Guzelian PS. 1984. Induction of cytochrome P-450 by glucocorticoids in rat liver. I. Evidence that glucocorticoids and pregnenolone 16 alpha-carbonitrile regulate de novo synthesis of a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver in vivo. *J Biol Chem* **259**: 1999-2006.

21) Kim H, Putt DA, Zangar RC, Wolf CR, Guengerich FP, Edwards RJ, Hollenberg PF, Novak RF. 2001. Differential induction of rat hepatic cytochromes P450 3A1, 3A2, 2B1, 2B2, and 2E1 in response to pyridine treatment. *Drug Metabolism Disposition* **29**: 353-360.

22) Chen ZY, White CC, He CY, Liu YF, Eaton DL. 1995. Zonal differences in DNA synthesis activity and cytochrome P450 gene expression in livers of male F344 rats treated with five nongenotoxic carcinogens. *J Environ Pathol Toxicol Oncol* **14**: 83-99.

23) Lake BG, Renwick AB, Cunninghame ME, Price RJ, Surry D, Evans DC. 1998. Comparison of the effects of some CYP3A and other enzyme inducers on replicative DNA synthesis and cytochrome P450 isoforms in rat liver. *Toxicology* **131**: 9-20.

24) Garg BD, Kovacs K, Tuchweber B, Khandekar JD. 1975. Effect of pregnenolone-16-alpha-carbonitrile, a microsomal enzyme inducer, on the regenerating rat liver. *Acta Anat (Basel)* **91**: 161-174.

25) Puntarulo S and Cederbaum AI. 1998. Production of reactive oxygen species by microsomes enriched in specific human cytochrome P450 enzymes. *Free Radical Biol Med* **24**: 1324-1330.

26) Debri K, Boobis AR, Davies DS, Edwards RJ. 1995. Distribution and induction of CYP3A1 and CYP3A2 in rat liver and extrahepatic tissues. *Biochem Pharmaco*l **50**: 2047-2056.

27) Pons F, Calvet JH, Haag M, Raeppel V, Keravis T, Frossard N. 2001. Altered expression of lung cytochrome P450 3A1 in rat after exposure to sulfur mustard. *Pharmacol Toxicol* **88**: 40-44

28) Ingold KU, Burton GW, Foster DO, Hughes L, Lindsay DA, Webb A. 1987. Biokinetics and discrimination between dietary RRR- and SRR-α-tocopherols in the male rat. *Lipids* **22**: 163-172.

29) Sato Y, Arai H, Miyata A, Tokita S, Yamamoto K, Tanabe T. 1993. Primary structure of α-tocopherol transfer protein from rat liver. Homology with cellular retinaldehyde-binding protein. *J Biol Chem* **268**: 17705-17710.

30) Yoshida H, Yusin M, Ren I, Kuhlenkamp J, Hirano T, Stolz A, Kaplowitz N. 1992. Identification, purification, and immunochemical characterization of a tocopherol-binding protein in rat liver cytosol. *J Lipid Res* **33**:343-350.

31) Kayden, H.J., and Traber, M.G. 1993. Absorption, lipoprotein transport, and regulation of plasma concentration of vitamin E in human. *J Lipids Re.* **34**: 343-358.

32) Traber MG, Sokol RJ, Burton GW, Ingold KU, Papas AM, Huffaker JE, Kayden HJ. 1990. Impaired ability of patients with familial isolated vitamin E deficiency to incorporate alpha-tocopherol into lipoproteins secreted by the liver. *J Clin Inves.* **85**: 397-407.

33) Traber MG. 1997. Regulation of human plasma vitamin E. *Adv Pharmacol* **38**:49-63.

34) Ben Hamida M, Belal S, Sirugo G., Ben Hamida C, Panayides K, Ionannou P, Beckmann J, Mandel JL, Hentati F, Koenig M.1993. Friedreich's ataxia phenotype not linked to chromosome 9 and associated with selective autosomal recessive vitamin E deficiency in two inbred Tunisian families. *Neurology* **43**: 2179-2183.

35) Terasawa, Y., Ladha, Z., Leonard, S.W., Morrow, J.D., Newland, D., Sanan, D., Packer, L., Traber, M.G., and Farese, R.V. Jr. 2000. Increased atherosclerosis in hyperlipidemic mice deficient in alpha -tocopherol transfer protein and vitamin E. *Proc Natl Acad Sci USA* **97**: 13830-13834.

36) Leonard SW, Terasawa Y, Farese RVJ, Traber MG. 2002. Incorporation of deuterated RRR- or all rac α-tocopherol into plasma and tissues of α-tocopherol transfer protein null mice. Am *J Nutr* **75**: 555-560.

37) Ban R, Takitani K, Kim HS, Murata T, Morinobu T, Ogihara T. 2002. α-Tocopherol transfer protein expression in rat liver exposed to hyperoxia. *Free Radical Res.* **36**:933-938.

38) Wu CG, Hoek FJ, Groenink M, Reitsma PH, van Deventer SJ, Chamuleau RA. 1997. Correlation of repressed transcription of alpha-tocopherol transfer protein with serum alpha-tocopherol during hepatocarcinogenesis. *Int J Cancer* **71**: 686-690.

39) Takenaka A, Kita A, Ikeya M, Arai H, Igarashi K. 2007. Galactosamine-induced acute liver injury in rats reduces hepatic α-tocopherol transfer protein production. *J Nutr Sci Vitaminol* **53**: 366-371.

40) Parker RS, Sontag TJ, Swanson JE. 2000. Cytochrome P4503A-dependent metabolism of tocopherol and inhibition by sesamin. *Biochem Biophys Res Commun* **277**: 531-534.

41) Ikeda S, Tohyama T, Yamashita K. 2002. Dietary sesame seed and its lignands inhibit 2, 7, 8-trimethyl-2(2'-carboxyethyl)-6-hydroxychroman excretion into urine of rats fed γ-tocopherol. *J Nutr* **132**: 961-966.

42) Schultz M, Leist M, Petrzika M, Gassmann B, Brigelius-Flohé R. 1995. Novel urinary metabolite of alpha-tocopherol, 2,5,7,8- tetramethyl- 2(2'- carboxyethyl)-6-hydroxychroman, as an indicator of an adequate vitamin E supply? *Am J Clin Nutr* **62** (6 Suppl): 1527S-1534S.

43) Lodge JK, Riddlington J, Leonard S, Vaule H, Traber MG. 2001. Alpha- and gamma-tocotrienols are metabolized to carboxyethyl-hydroxychroman derivatives and excreted in human urine. *Lipids* **36**: 43-48.

44) Mantha SV, Prasad M, Kalra J, Prasad K. 1993. Antioxidant enzymes in hypercholesterolemia and effects of vitamin E in rabbits. *Atherosclerosis* **101**: 135-144.

45) Marcus SR, Chandrakala MV, Nadiger HA. 1993. Interaction between vitamin E and glutathione in rat brain-effect of alcohol administration. *J Nutr Biochem* **4**: 336-340.

46) Eder, K., Flader, D., Hirche, F., and Brandsch, C. 2002. Excess dietary vitamin E lowers the activities of antioxidative enzymes in erythrocytes of rats fed salmon oil. *J Nutr* **132**:3400-3404.

47) Cowan DB, Weisel RD, Williams WG, Mickle DAG.. 1993. Identification of oxygen responsive elements in the 5'-flanking region of the human glutathione peroxidase gene. *J Biol Chem* **268**: 26904-26910.

48) Bozzi A, Mavelli I, Finazzi A, Strom R, Wolf AM, Mondovi B, Rotilio G. 1976. Enzyme defense against reactive oxygen derivatives. II. Erythrocytes and tumor cells. *Mol Cell Biochem* **10**: 11-16.

49) Sato K, Ito K, Kohara H, Yamaguchi Y, Adachi K, Endo H. 1992. Negative regulation of catalase gene expression in hepatoma cells. *Mol Cell Biol* **12**: 2525-2533.

50) Taniguchi M, takeuchi T, Nakatsuka R, Watanabe T, Sato K. 2004. Molecular process in acute liver injury and regeneration induced by carbon tetrachloride. *Life Sci* **75**: 1539-1549.

51) Gong H, Singh SV, Singh SP, Mu Y, Lee JH, Saini SPS, Toma D, Ren S, Kagan VE, Day BW, Zimniak P, Xie W. 2006. Orphan nuclear receptor pregnane X receptor sensitizes oxidative stress responses in transgenic mice and cancerous cells. *Mol Endoocrinol* **20**: 279-290.

Table 1. Liver weight and other organ weights in rats fed of two levels of dietary vitamin E with PCN or DMSO administration.

	Organ weight *(g)*				
	Liver	Kidney	Lung	Heart	Testis
C50	7.54±0.56	1.94±0.09	1.22±0.15	0.83±0.06	2.44±0.27
P50	10.15±1.59	1.87±0.19	1.21±0.21	0.8±0.09	2.51±0.28
C500	7.91±1.32	2.03±0.25	1.37±0.29	0.82±0.11	2.5±0.22
P500	10.72±1.67	2.02±0.17	1.37±0.19	0.8±0.06	2.49±0.16
Vitamin E	0.3246	0.0709	0.0436	0.9048	0.8228
PCN	<.0001*	0.5926	0.9245	0.3085	0.7215
Vitamin E×PCN	0.8238	0.6907	0.9194	0.9232	0.6104

1. C50, P50: rats fed a diet containing 50 ppm of α-tocopherol acetate injected with DMSO (C50) or PCN (P50). C500, P500: rats fed a diet containing 500 ppm of α-tocopherol acetate injected with DMSO (C500) or PCN (P500). n =8 for the C50 and P50 groups and n =9 for the C500 and P500 groups.

2. Each value is the mean±SD. Values not sharing common superscript are significantly different from one another among the four groups by one-way ANOVA and Duncan's multiple range test ($p<0.05$).

3. Analyzed by two-way ANOVA among the four groups. * denotes $p<0.05$.

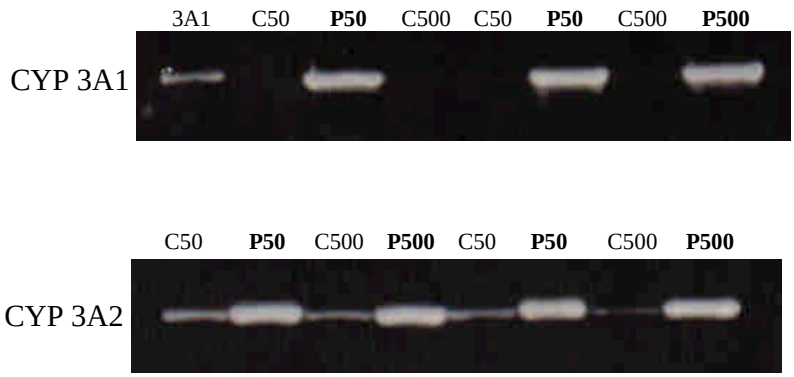


Figure 1 Western blot of CYP3A1 and CYP3A2 in livers of rats fed experimental diets after PCN or DMSO injection .

Table 2. Plasma and tissue α-tocopherol concentrations in rats fed of two levels of dietary vitamin E with PCN or DMSO administration.

	Plasma	Liver	Kidney	Lung	Heart	Testis
	(μmol/L)			*(nmol/g)*		
C50	16.5±3.5[b]	30.6±6.3 [bc]	27.81±2.58[b]	16.12±8.04[b]	17.61±5.66b[c]	39.06±2.89[b]
P50	10.4±1.1[c]	12.8±4.5 [c]	23.94±3.07[b]	17.88±6.32[b]	13.84±5.39[c]	29.62±2.92[b]
C500	26.5±3.5[a]	113.7±29.4 [a]	40.14±3.67[a]	35.89±18.09[a]	26.68±12.88[ab]	53.45±6.52[a]
P500	18.4±4.2[b]	40.2±23.3 [b]	37.95±7.16[a]	30.96±6.54[a]	29.74±14.52[a]	54.86±23.48[a]
Vitamin E	<0.0001*	<0.0001*	<0.0001*	0.0002*	0.0029*	0.0002*
PCN	<0.0001*	<0.0001*	<0.0679	0.6313	0.9910	0.4794
Vitamin E ×PCN	0.4024	0.0004*	<0.6034	0.4010	0.3759	0.2570

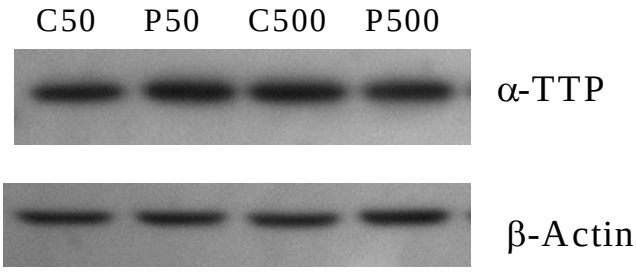
1. C50, P50: rats fed a diet containing 50 ppm of α-tocopherol acetate injected with DMSO (C50) or PCN (P50). C500, P500: rats fed a diet containing 500 ppm of α-tocopherol acetate injected with DMSO (C500) or PCN (P500). n =8 for the C50 and P50 groups and n =9 for the C500 and P500 groups.

2.Each value is the mean±SD.

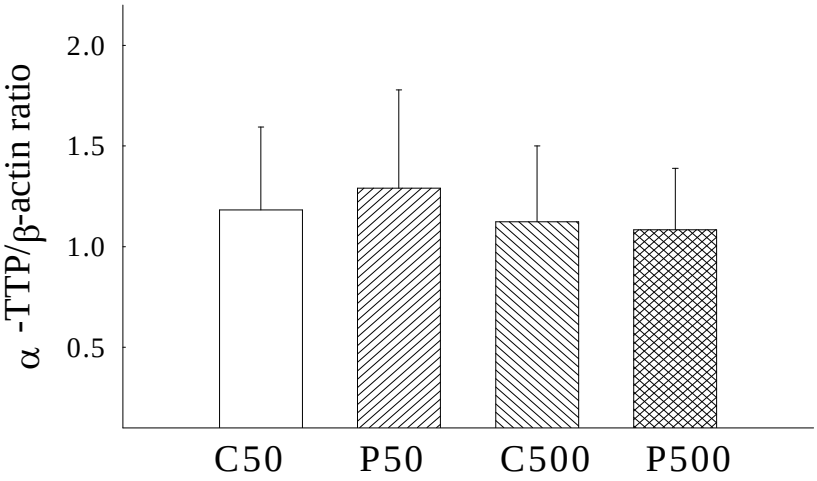
3.Analyzed by two-way ANOVA among the four groups. * denotes $p<0.05$.

4.Values not sharing common superscript are significantly different from one another among the four groups by one-way ANOVA and Duncan’s multiple range test ($p<0.05$).

A



B



C

Factor	Two-way ANOVA p value
Dietary vitamin E	0.3361
PCN	0.8288
Vitamin E×PCN	0.5976

Figure 2 Liver α-Tocopherol transfer protein levels analyzed by Western blot. (A) The original figure and (B) the ratio of each band intensity in values are means±SD and represented as bars. (C) The two-way ANOVA results of western blot analysis

Table 3. Tissue TBARS concentrations in rats fed two levels of dietary vitamin E with PCN or DMSO administration.

	Liver	Kidney	Lung	Heart	Testis
			(nmol/g liver)		
C50	118.4 ± 51.6 [b]	791±112	790±183	886±198	270±33
P50	308.3 ± 69.7 [a]	651±78	604±114	1000±292	265±54
C500	79.1 ± 9.0 [b]	697±87	743±233	825±197	236±24
P500	122.4 ± 46.8 [b]	719±144	587±127	727±123	237±20
Vitamin E	<0.0001*	0.7335	0.5431	0.0369*	0.0168*
PCN	0.0013*	0.1611	0.0072*	0.8765	0.8779
Vitamin E× PCN	0.0480*	0.0395*	0.7188	0.1165	0.7894

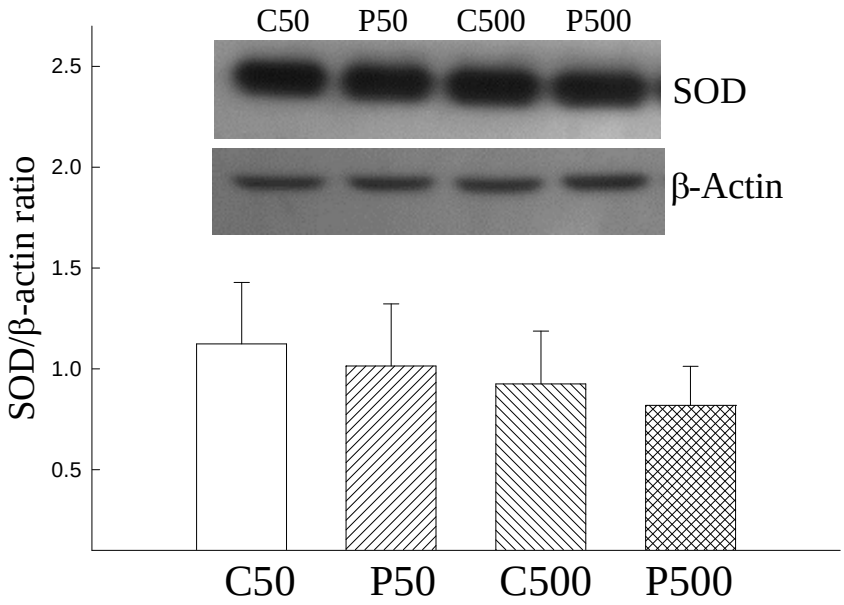
1. C50, P50: rats fed a diet containing 50 ppm of α-tocopherol acetate injected with DMSO (C50) or PCN (P50). C500, P500: rats fed a diet containing 500 ppm of α-tocopherol acetate injected with DMSO (C500) or PCN (P500). n =8 for the C50 and P50 groups and n =9 for the C500 and P500 groups.

2.Each value is the mean±SD.

3.Analyzed by two-way ANOVA among the four groups. * denotes $p<0.05$.

4.Values not sharing common superscript are significantly different from one another among the four groups by one-way ANOVA and Duncan's multiple range test ($p<0.05$).

A

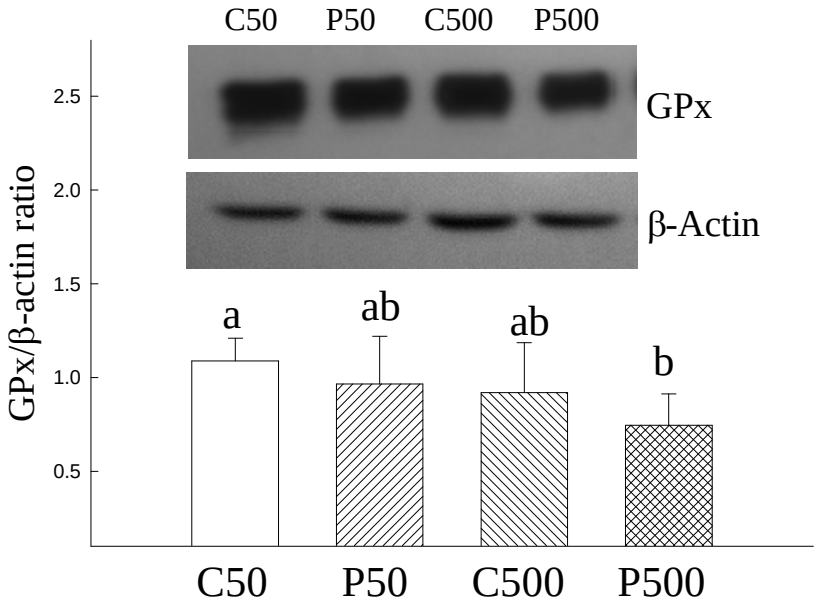


B

Factor	Two-way ANOVA p value
Dietary vitamin E	0.0424*
PCN	0.2512
Vitamin E×PCN	0.9805

Figure 3 The Western blot of SOD protein levels in livers of rats. (A) The original figure and the ratio of each band intensity in values are means±SD and represented as bars. (B) The two-way ANOVA results of western blot analysis of SOD (* indicates $p<0.05$).

A

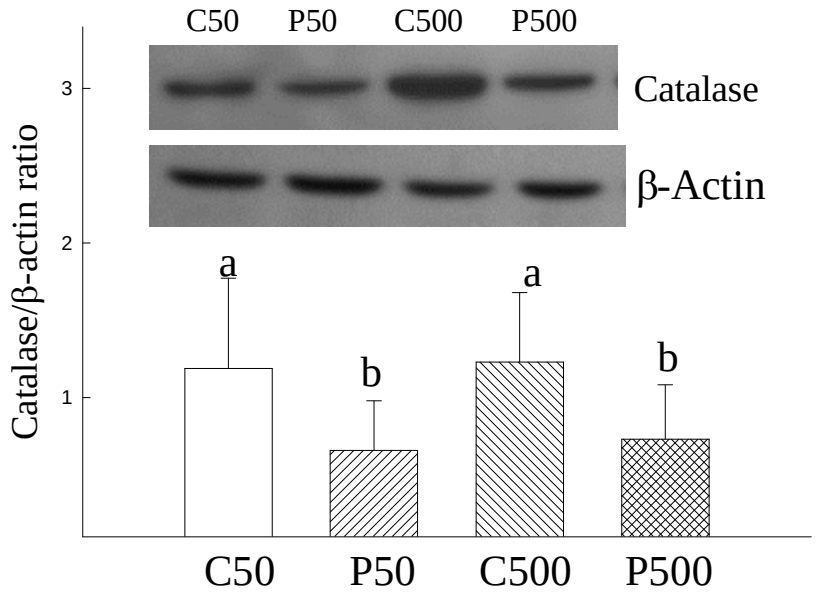


B

Factor	Two-way ANOVA p value
Dietary vitamin E	0.0120*
PCN	0.0462*
Vitamin E×PCN	0.7370

Figure 4 The Western blot of GPx protein levels in livers of rats. (A) The original figure and the ratio of each band intensity in values are means±SD and represented as bars. Bars not sharing at least one letter are significantly different from one another ($p<0.05$) (B) The two-way ANOVA results of western blot analysis of GPx (* indicates $p<0.05$).

A



B

Factor	Two-way ANOVA p value
Dietary Vitamin E	0.6211
PCN	0.0019*
Vitamin E×PCN	0.9149

Figure 5 The Western blot of Catalase protein levels in livers of rats. (A) The original figure and the ratio of each band intensity in values are means±SD and represented as bars. Bars not sharing at least one letter are significantly different from one another ($p<0.05$) (B) The two-way ANOVA results of western blot analysis of Catalase (* indicates $p<0.05$).