Effects of Vitamin E supplement and Perilla oil on the Cytochrome P-450 contents and Fatty acid composition in Rat Hepatocarcinogenesis

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ABSTRACT

The effects of vitamin E supplement on 15%(w/w diet) perilla or corn oils were studied in rat hepatocellular chemical carcinogenesis induced by modified Solt & Farber model, which consists of 20mg/kg body weight diethylnitrosamine(DEN) injection, 3 weeks feeding of 0.02%2-acetylaminofluorene(2-AAF) and partial hepatectomy. The area of placental glutathione S-transferase(GST-P) positive foci tended to be smaller in perilla oil than in corn oil groups. Despite of higher polyunsaturated fatty acid(PUFA) contents, the perilla oil group had lower thiobarbituric acid reactive substances(TBARS) content. Fatty acid compositions in microsomal membrane were reflected by dietary fatty acid compositions, and not affected by carcinogen treatment or vitamin E supplement. By vitamin E supplement, linolenic acid contents of perilla oil group were much increased. By carcinogen treatment, membrane stability decreased significantly in corn oil, but maintained in perilla oil groups. Vitamin E supplemental effect was noticed only in the corn-carcinogen group. Perilla oil may prevent hepatocarcinogenesis by maintaining membrane stability and by reducing cytochrome P-450 content. Vitamin E supplement did not seem to have the effect on hepatocarcinogenesis, but prevented lipid peroxidation, reduced cytochrome P-450 content and maintained membrane stability.

Key Words: Vitamin E, Perilla oil, Corn oil, Placental glutathione S-transferase positive foci, cytochrome P-450

INTRODUCTION

With the economic development and the westernization of diets, the incidence of diabetes mellitus, coronary heart disease, and cancer has increased. Cancers are developed by the combination of genetic and environmental factors and 80 to 90 percent of their incidence is connected with the environmental factors. Therefore most cancers are considered to be preventable and about 35 percent of cancers are related to dietary intakes1) and increased intakes of vitamins and micronutrients may help prevent some cancers2). Epidemiological data suggested that the incidence of some cancers was low when people consumed foods rich in vitamin A, C and E, but high when they took less than optimal. Vitamin E is an anti-oxidant, so it can function as a anticarcinogen, a free radical scavenger. Vitamin E as a free radical scavenger neutralizes a free radical, so protects the phospholipids of the cell membrane, from oxidative stress3). Odeleye et al4) showed that animals supplemented with vitamin E had a low level of lipid peroxide and tumors of significantly small sizes. Smith et al.5) reported that animals administered by carcinogen and α-tocopherol showed less amount of malondialdehyde(MDA) and cytotoxicity in hepatocarcinogenesis. Vitamin E protects lipids from peroxidation, and maintains the polyunsaturated fatty acids(PUFA) of the cell membrane by quenching free radicals6,7). The phospholipids in the cell membrane consist of fatty acids with C16-22 . Saturated fatty acids and monounsaturated fatty acids(MUFA) are bonded in Sn1, and PUFA in Sn2. If the balance of n-3/n-6 in PUFA is broken, it will lead to the change of the membrane physiology and the activity of the membrane-bounded enzymes8). It will be followed by the structural and local motional changes, which will bring about the change of interaction between proteins and lipid, and between the binder and the acceptor. Among the enzymes, ATPase, adenylate cyclase, and drug metabolizing enzymes are sensitive to these changes. According to Hammor and Willis9), α-tocopherol helps maintain the integrity of the microsomal membrane by acting as an antioxidant. In the hepatic microsome, arachidonic acid interacts with vitamin E to keep the membrane structure intact and controls the insertion of PUFA. Thus, the higher the intake of PUFA, the more the vitamin E is required to prevent lipid peroxidation and maintain the normal function7). The Solt & Farber model10), one of the ways to induce hepatocarcinogenesis in animals, consists of diethylnitrosamine(DEN), 2-acetylaminofluorene (2-AAF), and partial hepatectomy: DEN is a strong hepatoma initiator, 2-AAF is a selective growth promoter. These DEN and AAF need the help of drug metabolizing enzymes for detoxification. In a process of substrate oxidation by use of oxygen, these enzymes produce free radicals, which attack the PUFA in the microsomal membrane to make lipid peroxides. The lipid peroxides interact with the hepatic microsomal membranes, proteins and DNA in cells. The interaction affects the structure and function of

the microsomal membrane, changing the activity of membrane-bounded enzymes. The purpose of this study was to determine the influences of dietary fats and vitamin E supplement on placental glutathione S-transferase(GST-P) positive foci, hepatic cytochrome P-450 contents and fatty acid composition of microsomal membrane in rats treated by chemical carcinogen.

MATERIALS AND METHODS

1. Animal and diet

Weanling Sprague-Dawley strain male rats weighing 40-80 g were used. Hepatocarcinogenesis was induced by Solt & Farber modification which consists of 200mg DEN/kg body weight injection, 3 week-feeding of 0.02% 2-AAF and partial hepatectomy. Rats were fed corn oil, rich in n-6 fatty acids, or perilla oil, rich in n-3 fatty acids at 15%(w/w) of diet with vitamin E at 100(This is the normal content of vitamin E in corn oil) and 1,000 IU/kg diet for 10 weeks. In control groups, saline used instead of DEN and sham operation was carried out. The rats were fasted for 12 hours before decapitation. Livers were weighed, samples of the left, median and right lobes were removed for histologic examination and the remainder was centrifuged to separate the microsomal and cytosolic fractions. The samples were quickly frozen with liquid nitrogen for experimental analysis.

2. Immunohistochemical analysis

Samples were fixed in 10% phosphate buffered formalin solution for hematoxlin-eosin staining and in cold acetone for immunohistochemical staining. Immunohistochemical analysis was carried out with sequential treatments of rabbit-anti placental glutathione S-transferase (GST-P) antibody as a primary antibody (1:300), swine anti rabbit IgG antibody (1:30) as a second antibody and avidin biotin peroxidase complex(ABC) (1:300)11). Final visualization of the GST-P positive foci was enzymatically activated by 3,3-diaminobenzidine and H2O2 as substrates. The area of the GST-P positive foci were measured by image analyzer.

3. Biochemical analysis

Lipids were extracted according to the Bligh & Dyer method12) and analyzed the fatty acid composition by gas chromatography to investigate whether the interaction of n-6/n-3 fatty acids and the vitamin E supplement affects the microsomal membrane. The contents of cytochrome P-450 in microsome were analyzed by the method of Omura & Sato13), activities of NADPH-cytochrome P-450 reductase by the method of Master et al.14), thiobarbituric acid reactive substances(TBARS) contents by the method of Vaca & Harm-Ringdahl15), glucose 6-phosphatase by Baginski et al.16), serum γ-glutamyl transpeptidase(GGT) by Wako kit(code no. 271-56909). The Lowry's method17) was used to measure protein contents in microsomes and cytosol, using bovine serum albumine as a standard solution. Data were analyzed by SAS and the statistical comparison were made by Duncan's multiple range test to each group. The coefficients of correlation were calculated to find out how three factors of dietary fats, vitamin E supplement, carcinogen treatment affect biochemical indices.

RESULTS AND DISCUSSIONS

The weights of body, liver and the liver/body weight ratio were measured. After injection of DEN and partial hepatectomy, the average weight of carcinogen treated groups was lower than that of the control groups. There was slower weight gain in carcinogen treated groups. The weight gains were not affected by dietary fats and vitamin E supplement. The area of GST-P positive foci was detected only carcinogen treated groups. Perilla oil groups seemed to have reduced area of placental GST-P positive foci than corn oil groups. Vitamin E supplement did not change the area of GST-P positive foci at 1,000 IU, but by our previous study18) 15,000 IU vitamin E/kg diet significantly decreased the area of GST-P positive foci. Fatty acid compositions of microsomal membrane were reflected by dietary fats. Linoleic acid contents of corn oil groups was not significantly different from those of perilla oil groups. By carcinogen treat, linoleic acid contents of corn oil groups were increased. Linolenic acid contents of perilla oil were higher than corn oil groups and increased even higher by vitamin E supplement. Arachidonic acid contents of corn oil groups were higher than those of perilla oil groups, but the difference was not significant. Eicosapentaenoic acid(EPA) contents of perilla oil group was high, because n-3 PUFA in perilla oil is thought to convert to EPA. Despite the higher p/s ratio of perilla oil(7.3), p/s ratio of microsomal membrane was not different by dietary fats and vitamin E supplement. This result coincided with

McMurchie et al.19), who showed that the p/s ratio of membrane phospholipids was not changed when the animals were fed on diets with different lipid characters. Cytochrome P-450 contents were higher both in control and carcinogen treated corn oil groups. The result was coincident with the research of Wade and Norred20). that the diet supplied with 10% corn oil increased the cytochrome P-450. Cerutti21) showed that the more intakes of fish and vegetable oils rich in linolenic acid, the higher contents of cytochrome P-450. It is thought that the increase of linolenic acid and arachidonic acids by the intake of linoleic acid stimulates the metabolism of drugs9). In control groups, vitamin E supplement decreased the content of cytochrome P-450, particular in corn oil groups. Dashman and Kamm22) reported that the vitamin E supplement decreased the activities of mixed function oxidase(MFO) including cytochrome P-450 contents in hepatic cells. Our result suggests that vitamin E supplement in control group may protect the liver damage from the toxic substances, which was induced by the high contents of cytochrome P-45023). NADPH-cytochrome P-450 reductase could produce free radicals in the detoxification process of activated external drugs. The formation of free radicals is the initial step of NADPH-dependant lipid peroxidation and the product enhanced the lipid oxidation24). In our experiments, the activity of NADPH-cytochrome P-450 reductase was increased significantly by the carcinogen treatment($P<0.001$), not affected by the dietary fats and vitamin E supplement. It shows the positive correlation with the content of cytochrome P-450($r = 0.47$, $P<0.05$), and the production of lipid peroxide in microsomal membrane($r = 0.72$, $P<0.001$). The thiobarbituric acid reactive substance(TBARS) contents in microsomal fraction were higher in carcinogen treated groups than in the control groups. In the control groups, vitamin E supplement decreased the TBARS regardless of dietary fats. On the other hand, in the carcinogen treated groups, vitamin E supplement decreased the TBARS only in the corn oil group. In spite of the higher p/s ratio of perilla oil than that of corn oil, the contents of TBARS did not show significant difference in two groups. It suggests that vitamin E supplement protects the phospholipids in microsomal membrane, so the production of lipid peroxidation decreases4, 5, 18, 25) and the vitamin requirement may depend not on the P/S ratio but on types of dietary fats. The recent studies showed the production of TBARS and free radicals were increased, because the balance of production and clearance in lipid peroxide was broken. Glucose 6-phosphatase(G6Pase) activities, which represents the stability of the cell membrane, has a negative correlation with the TBARS($r = -0.64$, $P<0.001$). The activities of G6Pase were decreased when the cell membrane are attacked or tumors are developed16, 26). In our experiments, the activities of G6Pase were lowered by carcinogen treatment, much decreased in corn oil than perilla oil groups. The change of GGT activities is one of the indices of neoplasia of liver. Their activities in serum markedly increased when the liver is damaged by alcohols and by drugs. Our data shows the GGT activities in

serum increased by carcinogen treatment, not changed by the dietary fats and vitamin E supplements.

CONCLUSIONS

The area of GST-P positive foci tended to be smaller in perilla oil than in corn oil groups, but vitamin E supplement did not decrease the foci area. Despite of higher PUFA contents, the perilla oil groups had lower TBARS contents. The fatty acid compositions in microsomal membrane were reflected by dietary fatty acid compositions, and not affected by carcinogen treatment. Vitamin E supplement had perilla oil group's linolenic acid content increase. G6Pase activities, which represents the stability of the cell membrane, has a negative correlation with the contents of TBARS($r=-0.64$, $p<0.001$). By carcinogen treatment membrane stability maintained in perilla oil groups but decreased significantly in corn oil. This study suggests that effect of vitamin E supplement in hepatocarcinogenesis depends on the composition of dietary n-3 or n-6 fatty acids. Vitamin E supplemental effect was noticed only in the corn-carcinogen group. Perilla oil may prevent hepatocarcinogenesis by maintaining membrane stability and by reducing cytochrome P-450 content. Vitamin E supplement may prevents lipid peroxidation, reduces cytochrome P-450 content, and maintains membrane stability, but it did not seemed to have the same effect on hepatocarcinogenesis.

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REFERENCE

1) Newberne PM, Suphakarn V. Nutrition and Cancer : A review with emphasis on the role of vitamin C and E and selenium. Nutr Cancer 1983; 5: 107.

2) Watson RR. Selenium and vitamins A, E and C : nutrients with cancer prevention properties. J of Am Diet Assoc 1986; 86: 505-510.

3) Odin AP. Vitamins as antimutagens: advantages and some possible mechanisms of antimutagenic

action. Mutat Res 1997; 386(1): 36-67.

4) Odeleye OE, Eskeison CD, Mufti SI, Watson RR. Vitamin E inhibition of lipid peroxidation and ethanol-mediated promotion of esophageal tumorigenesis. Nutr Cancer 1992; 17: 223-234.

5) Smith MT, Thor H, Orrenius S. The role of lipid peroxidation in the toxicity of foreign compounds to liver cells. Biochem Phar 1983; 32(5): 763-764.

6) Packer L. Protective role of vitamin E in biological system. Am J Clin Nutr 1991; 53: 1050-1055.

7) Horwitt MK. Interpretations of requirement for thiamin, riboflavin, niacin-tryptophan and vitamin E plus comments on balance studies and vitamin B6. Am J Clin Nutr 1986; 44: 973-985.

8) Stubbs C, Smith AD. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim Biophy Acta 1984; 779:89-137.

9) Hammor CT, Wills ED. The effect of dietary fats on the composition of the liver endoplasmic reticulum and oxidative drug metabolism. Br J Nutr 1979; 41:465.

10) Solt D, Farber E. New principles for the analysis of chemical carcinogenesis. Nature 1976; 263: 701-703.

11) Osigo T, Tatematsu M, Tamano S, Tsuda H, Ito N. Comparative effects of carcinogenesis on the induction of placental glutathione S-transferase positive liver nodules in a short-term assay and of hepatocellular carcinogenesis in a long-term assay. Toxicol Pathol 1985; 13: 257-265.

12) Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37:911-917.

13) Omura T, Sato R. The carbon monooxide-binding pigment of liver microsomes. J of Biological Chemistry 1964; 239: 2370-2378.

14) Master BSS, Williams CG, Kamin H. The preparation and properties of TPHN-cytochrome C reductase from pig liver. Meth. Enzymol 1967; 10: 551-573.

15) Vaca CE, Harms-Ringdahl M. Lipid peroxidation in the rat liver S9 fraction: Influence of membrane lipid composition. Mutat Res 1986; 162:211-32.

16) Baginski ES, Foa PP, Zak B. Glucose 6-phosphatase. In: Method of enzymatic analysis. ed Bergmeyer 1983; 2: 876-880.

17) Lowry OH, Roserbrough NJ, Farr A L, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265-275.

18) Kim S, Kim J, Choi H. Suppressive Effects of Vitamin E on the induction of placental glutathione S-transferase(GST-P) positive foci and antioxidant enzyme activity in rat hepatocarcinogenesis. Korean J of Nutrition 1997; 30(7): 803-812.

19) McMurchie EJ, Abeywardena MY, Charnock JS, Gibson RA. The effect of dietary lipids on the thermetropic behaviour of rat liver and heart mitochondrial membrane lipids. Biochem Biophys Acta 1983; 734: 114-124.

20) Wade AE, Norred WP. Effect of dietary lipid on drug-metabolizing enzyme. Federation Proc 1979; 35: 2475-2479.

21) Cerutti PA. Prooxidant states and tumor promotion. Science 1985; 227: 375-381.

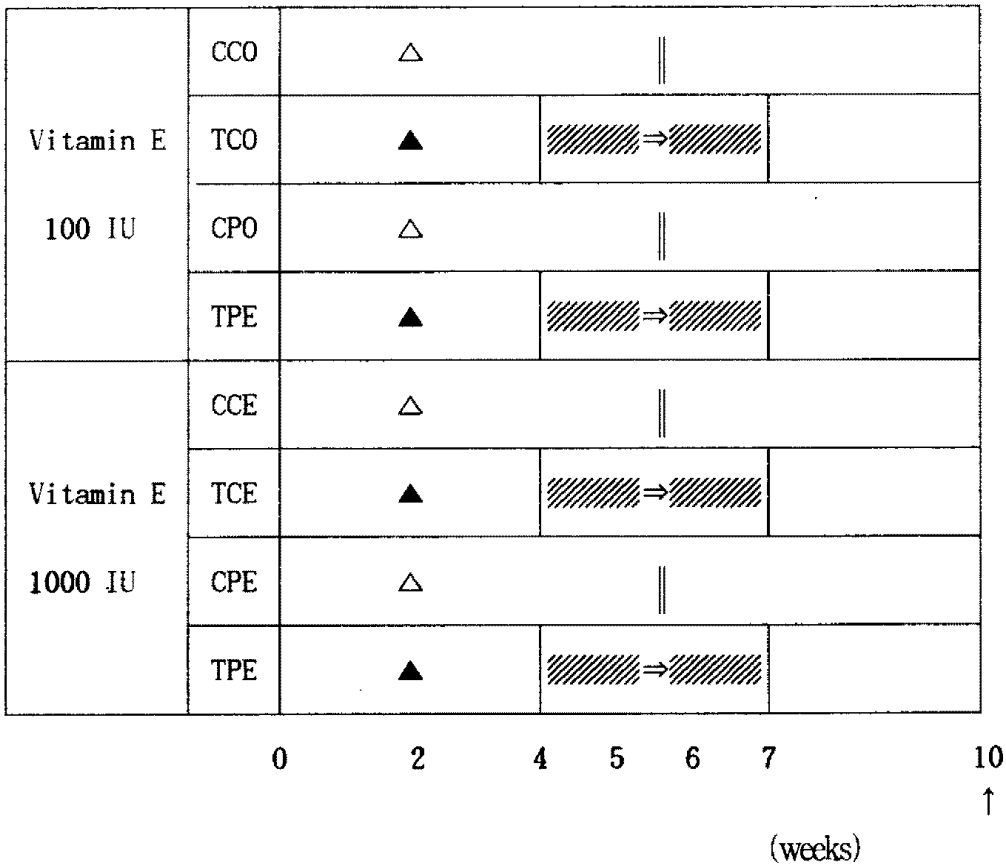
22) Dashman T, Kamm JJ. Effect of high doses of vitamin E on dimethylnitrosamine hepatotoxicity and drug metabolism in the rat. Biochem Pharmacol 1979; 28:1485-1490.

23) Bridges JW, Benford DJ, Rubbard SA. Mechanism of toxic injury. Ann Acad Sci 1983; 407: 42-63.

24) Lai C, Pietie LH. Hydroxyl radical production involved in lipid peroxidation of rat liver microsomes. Biochem Biophys Res Commu 1977; 78: 51059.

25) Kim S, Choi H. Effects of n-6, n-3 fatty acids and vitamin E supplement on the induction of preneoplastic lesion in murine hepatocarcinogenesis model. Korean Biochem J 1994; 27(2): 125-131.

26) Eriksson LC, Ulla-Britta T, Andersson GN. Isolation and characterization of endoplasmic reticulum and golgi apparatus from hepatocyte nodule in male Wistar rats. Cancer Res 1984; 43: 3335-3347.



△ 2.0 ml saline/kg B.W. as control

▲ 200 mg DEN in 2.0 ml saline/kg B.W i.p. injection

‖ sham operation

⇒ 70% partial hepatectomy

↑ Sampling

▨ + 0.02% AAF

CCO : control, corn oil(CO) group

CCE : control, corn oil group supplemented w/ 1,000 IU vit.E

TCO : carcinogen treated, corn oil group

TCE : carcinogen treated, corn oil group supplemented w/ 1,000 IU vit.E

CPO : control, perilla oil(PO) group

CPE : control, perilla oil group supplemented w/ 1,000 IU vit.E

TPO : carcinogen treated, perilla oil group

TPE : carcinogen treated, perilla oil group supplemented w/ 1,000 IU vit.E

〈Figure 1〉 Experimental design

Table 1. Composition of experimental diet (g/100g diet)

Ingredient/Diet	CO	CE	PO	PE
Corn starch	54.7	54.7	54.7	54.7
Casein	20.0	20.0	20.0	20.0
Cellulose	5.0	5.0	5.0	5.0
Salt mixture(1)	4.0	4.0	4.0	4.0
Vitamin mixture(2)	1.0	1.0	1.0	1.0
DL-Methionine	0.3	0.3	0.3	0.3
Corn oil	15.0	15.0	-	-
Perilla oil	-		15.0	15.0
α-Tocopherol acetate(3)	-	0.9	0.04	0.94

CO : corn oil diet

CE : corn oil diet supplemented w/ 1,000 IU vitamin E

PO : perilla oil diet

PE : perilla oil diet supplemented w/ 1,000 IU vitamin E

(1) Composition of salt mixture, g/kg mixture : CaHPO4 500g, NaCl 74g, K2SO4 52g, Potassium Citrate Monohydrate 220g, MgO 24g, Manganese Carbonate (43-48 Mn) 3.5g, Ferric Citrate (16-17% Fe) 6.0g, Zinc Carbonate 1.6g, Cupric Carbonate (53-55% Cu) 0.3g, KIO3 0.01g, Chromium Potassium Sulfate 0.55g, Na2SeO3. 5H2O 0.01g, Sucrose finely powdered 118.0g

(2) Nutritional Biochemicals, ICN Life Science Group, Cleveland, Ohio. Vitamin mixture is composed of : Vit.A Acetate (500,000 IU per g) 0.125, α-Tocopherol (250 IU per g) 22.0g, Ascorbic acid 45.0g, Inositol 5.0g, Choline Chloride 75.0g, Menadione 2.25g, p-Aminobenzoic acid 5.0g, Niacin 4.25g, Riboflavin 1.0g,pyridoxine hydrochloride 1.0g, Calcium Pantothenate 3.0g, Biotin 0.02g, Folic acid 0.09g, Vit.B12 0.00135g and Dextrose to 1kg

(3) Sigma No. T 3376 DL-α-Tocopherol acetate

〈Table 2〉 Effects of vitamin E supplement and dietary fats on the final body weight, liver weight and liver/body weight ratios

Group		Final body weight (gm)	Final liver weight (gm)	Liver/body weight ratio (%)
Corn oil	CCO	380.0±27.4 ^{ab}	10.15±0.85	2.67±0.07
	CCE	396.7±12.3 ^{a}	10.11±0.72	2.54±0.10
	TCO	318.8±14.8 ^{bc}	9.30±0.58	2.92±0.14
	TCE	325.0±19.7 ^{bc}	9 12±0.79	2.79±0.12
Perilla oil	CPO	380.0±45.3 ^{ab}	9.62±1.43	2.50±0.08
	CPE	412.0±26.3 ^{a}	11.17±0.97	2.71±0.17
	TPO	291.0±15.2 ^{c}	8.89±0.71	3.05±0.16
	TPE	296.3±10.5 ^{c}	8.74±0.60	2.94±0.14

Values are mean±SE.

Means with the same letter are not significant different at P<0.05 by Duncan's multiple range test

〈Table 3〉 Effects of vitamin E supplement and dietary fats on the area and number of placental glutathione S-transferase hyperplastic nodules

	Placental Glutathione S-transferase	
	mm^2/cm^2	no/cm^2
TCO	17.06 ± 3.47	32.94± 4.19
TCE	16.54 ± 1.78	37.49 ± 4.48
TPO	13.14 ± 9.95	30.17 ± 5.20
TPE	11.77 ± 2.67	41.29 ±10.21

Values are mean±SE.

Means with the same letter are not significant different at P<0.05 by Duncan's multiple range test

Table 4. Effects of vitamin E supplement and dietary fats on the microsomal NADPH-cytochrome P-450 reductase, glucose 6-phosphatase, serum γ-glutamyltranspeptidase activities

Group		NADPH-cytochrome P-450 reductase nmole DCIP/mg protein	Glucose 6-phosphatase nmole Pi liberated/mg protein	γ-Glutamyltranspeptidase GGT unit/mg protein
Corn oil	CCO	45.77 ± 2.05^{b}	696.47 ± 24.12^{a}	0.86 ± 0.06^{c}
	CCE	53.28 ± 1.81^{b}	538.34 ± 27.79^{abc}	0.97 ± 0.09^{c}
	TCO	79.36 ± 5.62^{a}	262.68 ± 19.66^{c}	4.00 ± 0.62^{b}
	TCE	77.17 ± 5.63^{a}	331.97 ± 27.09^{bc}	5.04 ± 0.36^{b}
Perilla oil	CPO	49.02 ± 3.49^{b}	551.11 ± 56.26^{abc}	0.89 ± 0.04^{c}
	CPE	50.70 ± 4.81^{b}	574.06 ± 76.85^{ab}	1.08 ± 0.01^{c}
	TPO	75.37 ± 5.23^{a}	402.65 ±110.60^{abc}	4.54 ± 0.45^{b}
	TPE	85.83 ± 2.18^{a}	492.52 ± 89.01^{abc}	6.83 ± 0.61^{a}

Values are mean±SE.

Means with the same letter are not significant different at $P<0.05$ by Duncan's multiple range test

〈Table 5〉 Effects of vitamin E supplement on the microsomal fatty acid composition in corn oil diet fed rats

	Corn oil			
	CCO	CCE	TCO	TCE
16:0	28.77±3.49^{ab}	28.42±1.93^{ab}	27.08±0.90^{ab}	31.96±2.11^{a}
16:1	-	-	-	0.12±0.12^{a}
18:0	30.57±2.49^{a}	29.48±3.59^{a}	25.01±1.44^{a}	27.63±3.24^{a}
18:1	9.18±0.72^{a}	8.96±1.53^{a}	10.96±1.72^{a}	8.89±0.59^{a}
18:2	11.69±1.88^{b}	14.54±2.84^{ab}	19.62±1.92^{ab}	17.38±3.52^{ab}
18:3	0.69±0.47^{bc}	0.71±0.25^{bc}	0.81±0.65^{bc}	0.18±0.06^{c}
20:2	3.01±1.18^{a}	1.79±1.10^{a}	0.17±0.17^{a}	1.09±0.76^{a}
20:4	15.58±3.89^{a}	15.46±2.08^{a}	15.67±1.59^{a}	11.96±3.17^{a}
20:5	-	-	0.09±0.09^{b}	-
22:6	0.50±0.50^{a}	0.66±0.38^{a}	0.59±0.38^{a}	0.80±0.58^{a}
PUFA	40.65±4.25^{a}	42.10±3.61^{a}	47.91±1.54^{a}	40.42±3.64^{a}
SFA	59.35±4.25^{a}	57.90±3.61^{a}	52.09±1.54^{a}	59.58±3.64^{a}
P/S	0.71±0.13^{a}	0.75±0.11^{a}	0.92±0.06^{a}	0.70±0.10^{a}

CCO:Control, Corn oil diet
CCE:Control, Corn oil diet with Vitamin E supplement
TCO:Carcinogen treated, Corn oil diet
TCE:Carcinogen treated, Corn oil diet with Vitamin E supplement

Values are mean±SE.
Means with different letters are significantly different at $P<0.05$ by Duncan's multiple range test.

Table 6. Effects of vitamin E supplement on the microsomal fatty acid composition in perilla oil diet fed rats

	Perilla oil			
	CPO	CPE	TPO	TPE
16:0	23.51±3.12^{b}	22.29±2.52^{b}	27.14±1.68^{ab}	22.07±1.13^{b}
16:1	0.32±0.32^{a}	0.32±0.20^{a}	-	0.22±0.22^{a}
18:0	30.06±2.53^{a}	29.67±2.28^{a}	28.78±2.72^{a}	25.72±2.53^{a}
18:1	10.10±0.31^{a}	10.22±0.61^{a}	10.88±1.26^{a}	10.65±1.05^{a}
18:2	17.39±2.84^{ab}	16.23±1.97^{ab}	15.57±1.26^{ab}	19.85±2.16^{a}
18:3	3.69±0.29^{ab}	6.51±1.75^{a}	2.27±0.94^{b}	6.08±1.48^{a}
20:2	2.63±1.66^{a}	1.71±0.77^{a}	2.92±1.16^{a}	0.39±0.39^{a}
20:4	8.65±2.17^{a}	8.39±0.79^{a}	8.54±1.11^{a}	8.62±0.98^{a}
20:5	2.92±1.05^{a}	3.49±0.96^{a}	2.40±1.39^{ab}	4.23±0.90^{a}
22:6	0.73±0.59^{a}	1.17±0.78^{a}	1.50±0.87^{a}	2.18±0.76^{a}
PUFA	46.43±4.53^{a}	48.04±4.74^{a}	44.08±4.26^{a}	52.21±3.45^{a}
SFA	53.57±4.53^{a}	51.96±4.74^{a}	55.92±4.26^{a}	47.79±3.45^{a}
P/S	0.91±0.16^{a}	0.97±0.18^{a}	0.82±0.15^{a}	1.13±0.17^{a}

CPO: Control, Perilla oil diet
CPE: Control, Perilla oil diet with Vitamin E supplement
TPO: Carcinogen treated, Perilla oil diet
TPE: Carcinogen treated, Perilla oil diet with Vitamin E supplement

Values are mean±SE.
Means with different letters are significantly different at $P<0.05$ by Duncan's multiple range test.

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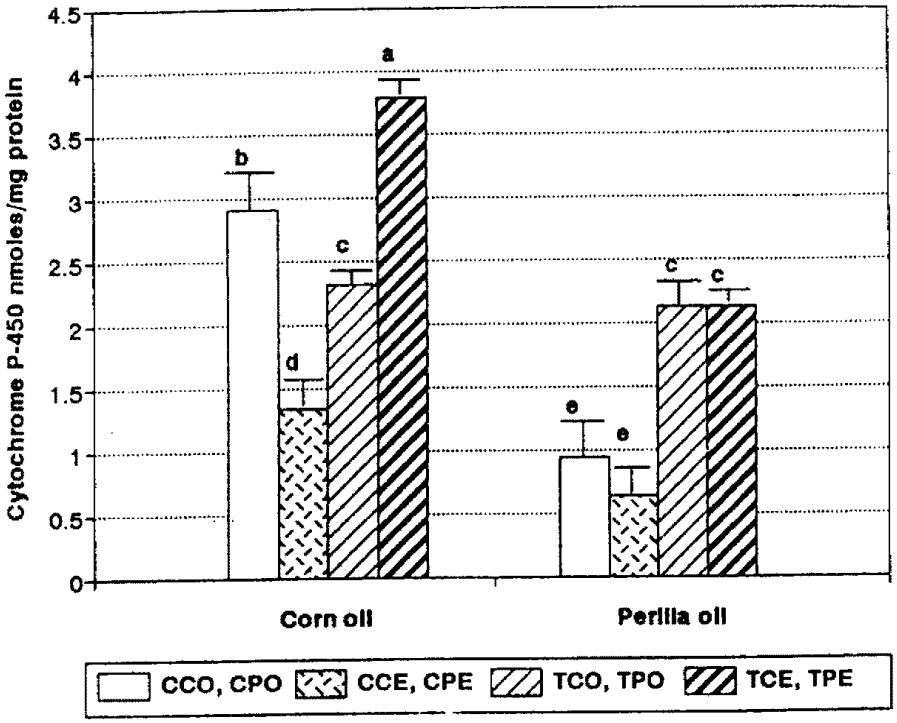

CCO : control, corn oil(CO) group
CCE : control, corn oil group supplemented w/ 1,000 IU vit.E
TCO : carcinogen treated, corn oil group
TCE : carcinogen treated, corn oil group supplemented w/ 1,000 IU vit.E
CPO : control, perilla oil(PO) group
CPE : control, perilla oil group supplemented w/ 1,000 IU vit.E
TPO : carcinogen treated, perilla oil group
TPE : carcinogen treated, perilla oil group supplemented w/ 1,000 IU vit.E

Values are mean±SE.
Means with the same letter are not significant different at $P<0.05$ by Duncan's multiple range test

〈Figure 2〉 Effects of vitamin E supplement and dietary fats on the microsomal cytochrome P-450 contents, thiobarbituric acid reactive substances contents and linolenic acid levels

국 문 초 록

Solt & Farber 모델로 유도한 다단계 암화과정에서 비타민 E가 보강된 옥수수기름과 들기름식이가 cytochrome P-450과 생체막 지방산 조성에 미치는 효과

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본 연구는 식이를 통한 비타민 E보충과 서로 다른 식이지방이 간 세포 암화과정에서 cytochrome P-450 함량과 생체막 지방산 조성에 미치는 효과에 관한 것이다. 이유한 Sprague-Dawley계 숫쥐를 발암원 처리군과 대조군으로 나누어 Solt & Farber 모델로 암화과정을 유도하였다. 실험식이는 옥수수기름과 들기름을 지방급원으로 이용하였고 각 기름군의 비타민 E함량은 식이 1 kg당 100 IU와 1,000 IU로 조정하였다. 간 세포암이 개시, 발전되는 과정 중 간 소포체막의 지방산 조성 변화를 살펴보기 위하여 소포체막의 지방산 분석을 실시하였으며 약물대사 효소계인 cytochrome P-450, NADPH-cytochrome P-450 reductase활성도를 측정하였다. 이들과 지질과산화물 생성과의 관계를 살펴보기 위하여 thiobarbituric acid reactive substance(TBARS) 함량을 측정하였고, 생체막 안정도의 지표인 glucose 6-phosphatase(G6Pase)활성도도 측정하였다. 그리고 간 세포의 손상정도는 혈청 γ-glutamyl transpeptidase(GGT)활성도로 알아보고자 하였다.

소포체막의 지방산 조성은 식이지방이 잘 반영되어 옥수수기름군은 arachidonic acid비율이 높았고, 들기름군은 linolenic acid 비율이 높았다. 약물대사 효소계 중 cytochrome P-450 함량은 옥수수기름군이 들기름군보다 높았고 두가지 식이지방군 모두 대조군에서는 비타민 E 보충군의 cytochrome P-450 함량이 낮았다. NADPH-cytochrome P-450 reductase와 혈청 GGT는 식이요인에 관계없이 발암원 처리시 활성도가 증가했다. 지질과산화물 함량(TBARS)은 모든 식이군에서 발암원 처리군이 대조군보다 유의적으로 높았으며 발암원 처리를 한 옥수수기름군에서는 비타민 E보충식이를 섭취했을 때 지질과산화물 함량이 유의적으로 낮아진 반면에 들기름군에서는 유의적인 차이는 없었다. G6Pase활성도는 발암원 처리에 의하여 낮아졌으며, 옥수수기름군에서 비타민 E 보충은 발암원에 의하여 G6Pase활성도가 유의적으로 낮아지는 것을 방지해 주었다. 지질과산화물 생성은 GGT와는 양의 상관관계, G6Pase와는 강한 음의 상관관계를 보여, 지질과산화물 생성은 간손상정도가 심하고, 생체막 안정도가 깨어질수록 증가하는 것을 보여주었다.

이상의 결과로 볼 때 식이지방의 불포화도에 비례하여 지질과산화물 생성이 증가하는 것은 아니며 식이를 통한 비타민 E보충은 대조군의 cytochrome P-450함량을 낮추는 동시에 지질과산화물 생성을 감소시킴으로써

산화적 손상으로부터 세포막을 보호해 주었다. 그리고 이러한 생체방어 효과는 들기름보다 옥수수기름군에서 더 크게 나타났다. 그러므로 암화과정에서 보충한 비타민 E효과는 식이지방의 불포화도보다는 지방산 종류에 따라 다르게 나타나는 것으로 생각된다.