Comparison of the Effect of Fish Oil and Corn Oil on Chemical-Induced Hepatic Enzyme-Altered Foci in Rats

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The effects of fish oil and corn oil diets on diethylnitrosamine initiation/phenobarbital promotion of hepatic enzyme-altered foci in female Sprague−Dawley rats were investigated. Groups of 12 rats were initiated with diethylnitrosamine (15 mg/kg) at 24 h of age. After weaning, they received diets containing either 13.5% fish oil plus 1.5% corn oil or 15% corn oil for 24 weeks. Rats fed fish oil had significantly greater liver weight, relative liver weight, spleen weight, and relative spleen weight than rats fed corn oil ($p < 0.05$). Hepatic phospholipid fatty-acid profile was significantly affected by the type of dietary lipid. The rats fed fish oil had significantly greater hepatic phospholipid 20:5 and 22:6 than rats fed corn oil; in contrast, the rats fed corn oil had significantly greater hepatic phospholipid 18:2 and 20:4 than rats fed fish oil ($p < 0.05$). Rats fed fish oil had significantly lower hepatic vitamin E and PGE_2 content but significantly greater hepatic lipid peroxidation than rats fed corn oil ($p < 0.05$). The hepatic levels of antioxidant enzymes (GSH reductase and GST) were significantly greater in rats fed fish oil than in rats fed corn oil ($p < 0.05$). Except for PGST-positive foci (foci area/tissue area), all the other foci parameters (GGT-positive foci area/tissue area, GGT-positive foci no./cm^2, GGT-positive foci no./cm^3, PGST-positive foci no./cm^2, and PGST-positive foci no./cm^3) measured in the fish oil group were 10−30% of those in the corn oil group ($p < 0.05$). Analyses of Pearson correlation coefficient revealed a positive correlation between hepatic GGT- or PGST-positive foci number (no./cm^2) and PGE_2 content ($r = 0.66$, $P = 0.01$; $r = 0.56$, $P = 0.02$, respectively) but a negative correlation between GGT- and PGST-positive foci (no./cm^2) and lipid peroxidation ($r = -0.8$, $P = 0.0006$; $r = -0.58$, $P = 0.01$, respectively), GSH/(GSH + GSSG) ratio ($r = -0.61$, $P = 0.05$; $r = -0.4$, $P = 0.14$, respectively), GSH reductase ($r = -0.75$, $P = 0.002$; $r = -0.53$, $P = 0.02$, respectively), and GST activities ($r = -0.65$, $P = 0.01$; $r = -0.44$, $P = 0.07$, respectively). Similar correlation between foci number (no./cm^3) and PGE_2, lipid peroxidation, GSH/(GSH + GSSG) ratio, GSH reductase, and GST activities were obtained. The results of this study show that dietary fish oil significantly inhibited hepatic enzyme-altered foci formation compared with corn oil in rats. These results suggest that the possible mechanisms involved in this process are the stimulation of hepatic detoxification system, changes in membrane composition, inhibition of PGE_2 synthesis, the enhancement of GSH-related antioxidant capacity, and the enhancement of lipid peroxidation by fish oil.

Keywords: *Lipids; enzyme-altered foci; PGE_2; detoxification system; antioxidant capacity; lipid peroxidation*

INTRODUCTION

In both human epidemiological studies and laboratory animal studies, dietary fat has been shown to influence the development of cancer at different sites. Type of dietary fat is an important determinant in cancer chemoprevention. Lipids rich in n-3 polyunsaturated fatty acids (PUFA) are potential candidates to decrease cancer in several organs (Reddy and Maruyama, 1986; Jurkowski and Cave, 1985; O'Conner et al., 1989). Fish oil is a fat from fish and has an appreciable amount of n-3 PUFA, namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), but a small amount of n-6 PUFA. In studies on Alaskan and Greenland Eskimos, the consumption of large amounts of marine products with high levels of n-3 PUFA in the diets were found and this was suggested to be the main reason for a low incidence of colon cancer (Blot et al., 1975; Bang et al., 1976). However, n-6 PUFA which consists of mainly linoleic acid, has been shown to promote the development of tumors in colon, mammary gland, and pancreas in animal studies (Reddy and Maruyama, 1986; Jurkowski and Cave, 1985; O'Conner et al., 1989; Onogi et al., 1996; Komaki et al., 1996). In our previous study (Chen et al., 1997a), type of dietary fat was shown to affect hepatic enzyme-altered foci formation after diethylnitrosamine (DEN) initiation and phenobarbital (PB) promotion in female Sprague−Dawley rats. However, the underlying mechanism of this effect is not known.

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Liver carcinogenesis is a multistage process that includes initiation and promotion (Faber, 1984; Pitot and Sirica, 1980). After initiation by a single administration of the representative complete carcinogen DEN (Goldsworthy and Pitot, 1985), the hepatopromoter PB has been shown to increase foci and neoplasms in rats (Pitot et al., 1977). Hepatic enzyme-altered foci can reflect clonal development from single initiated hepatocytes (Peraino et al., 1984; Weinberg et al., 1987; Goldsworthy et al., 1986), but no known histochemical markers can exactly assess the relationship between foci and tumors, and none of the markers invariably appears in all tumors (Peraino et al., 1984).

Inhibition of prostaglandin (PG) synthesis by PG-synthesis inhibitors is reported to suppress experimental chemical carcinogenesis in colon (Mernett, 1992), mammary (McCormick et al., 1985), pancreas (Takahashi et al., 1990), bladder (Shibata et al., 1993), and liver (Denda et al., 1989; Denda et al., 1994). Furthermore, PGs have been found to increase DNA synthesis in cultured hepatocytes (Hong and Glauert, 1996; Refsnes et al., 1994; Skouteris et al., 1988). High levels of PGs have been found in several animal and human tumors, particularly those of the E series which have been shown to be closely related to cell proliferation and tumor growth and to suppress immune responsiveness (Karmali, 1985). PGE_2 has been considered to play an important role in both human basal and squamous cell skin carcinomas because elevated levels of PGE_2 in skin carcinomas are associated with an aggressive growth pattern (Vanderveen et al., 1986). PGs of the 2 series are derived from dietary n-6 lipids. In contrast, PGs of the 3 series are derived from dietary n-3 lipids. The formation of 2-series PGs and related compounds from dietary n-6 lipids is catalyzed by cyclooxygenase (De Vries et al., 1992). EPA, an n-3 PUFA present in fish oil, can compete with arachidonic acid (AA) for cyclooxygenase and, thus, decrease the production of AA-derived compounds (Samuelsson et al., 1987). Compared with n-6 lipids, n-3 lipids have been shown to inhibit tumor development (Reddy and Maruyama, 1986; Jurkowski and Cave, 1985; O'Conner et al., 1989). The anticarcinogenic contribution of n-3 lipids may be ascribed to their inhibition of 2-series PG synthesis. In addition to PGs, lipid peroxidation has been speculated to be implicated in carcinogenesis as well. Slater (1988) found that experimental liver tumors express increased antioxidant capacity and a decreased potential for lipid peroxidation, and this condition has been suggested to favor the neoplastic growth of initiated cells (Dormandy and Wickens, 1988; Cerutti et al., 1988).

The present study investigated the effect of n-3 and n-6 lipids on hepatocarcinogenesis in terms of hepatic enzyme-altered foci formation in a DEN-initiation/PB-promotion model, and the possible mechanisms involved in this process. Two commonly used end points for the detection of preneoplastic hepatic lesions were used, the expression of the placental form of glutathione S-transferase (PGST) and γ-glutamyl transpeptidase (GGT).

MATERIALS AND METHODS

Animals and Diets. Pregnant Sprague−Dawley rats were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). To investigate the different experimental conditions on foci and tumor generation, Peraino et al. (1984) devised an experimental hepatocarcinogenesis protocol that produced a rapid induction of maximal yields of foci and tumors with minimal carcinogen treatment. In this study, we used the experimental protocol of Peraino et al. (1984). Female pups were initiated with 15 mg/kg DEN in phosphate buffered saline (pH 7.0) at 24 h of age by intraperitoneal injection. Twenty-four weaning rats were randomly assigned to two experimental diets and there were 12 rats in each group. The experimental diets were nutritionally complete and provided 30% of energy as fat (Table 1). Fish oil was obtained from Tama Biochemical Co. (Tokyo, Japan) and corn oil was obtained from CPC Intl. Inc. (Englewood, NJ). The α-tocopherol content of fish oil was 49 tocopherol equiv (TE)/100 g and that of corn oil was 17 TE/100 g. The two groups of rats were provided ad libitum access to feed and tap water for 24 weeks.

Table 1. Composition of Experimental Diets

ingredient	fish oil (%)	corn oil (%)
corn oil[a]	1.5	15.0
fish oil[b]	13.5	
casein[c]	15.0	15.0
dextrose[c]	15.0	15.0
corn starch	44.95	44.95
cellulose[c]	5.0	5.0
AIN76 vitamin mixture[c]	1.0	1.0
AIN76 mineral mixture[c]	3.5	3.5
choline[c]	0.2	0.2
DL-methionine[c]	0.3	0.3
phenobarbital[d]	0.05	0.05

[a] From CPC Intl Inc. (Englewood, NJ). [b] From Tama Biochemical Co. (Tokyo, Japan). [c] From Teklad (Madison, WI). [d] From Sigma Chemical (St. Louis, MO).

Table 2. Fatty Acid Composition of Dietary Fats

fatty acid	fish oil (%)	corn oil (%)
14:0	8.02	0.07
16:0	9.75	10.37
16:1	9.87	0.08
18:0	3.64	1.96
18:1n-9	7.75	26.29
18:2n-6	1.48	60.48
20:0	0.53	0.4
18:3n-3	1.04	
20:1	0.09	0.35
20:2	0.25	
20:3	0.19	
22:0	0.33	
20:4n-6	1.99	
22:1	0.06	
20:5n-3	37.01	
24:1	0.13	
22:6n-3	17.87	

Plasma Preparation. After 24 weeks of feeding, the rats were fasted overnight and killed by an overdose of CO_2. Blood for the PGE_2 assay was drawn from the jugular vein. 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 1500*g* for 5 min. Plasma was removed after centrifugation and stored at −80 °C for later analysis.

Lipid and Liver Phospholipid Fatty-Acid Profile Assay. The fatty acid composition of fish oil and corn oil was analyzed by gas chromatography (Table 2). Liver lipids were extracted according to the method of Folch et al. (1957). Total phospholipids were isolated from the lipid extracts by thin-layer chromatography as previously described by Chen et al. (1997). Fatty-acid analysis was performed as previously described by Lepage and Roy (1986). Fatty-acid methyl esters were quantified by gas chromatography using a 30-m fused-silica column with an internal diameter of 0.25 mm (Supelco, Bellefonte, PA). Peaks were identified by comparing retention times with those of authentic fatty-acid methyl ester standards (Alltech, Deerfield, IL). The percentage of each fatty acid was determined by integration of the peak areas.

Liver α-Tocopherol Level and Lipid Peroxidation Assays. Hepatic α-tocopherol concentration was determined as described by Catignani and Bieri (1983) with some modification (Lii et al., 1997). Briefly, 50 μL of an internal standard (α-tocopheryl acetate in ethanol) and hepatic homogenate (100 μL taken from 0.1 g liver:1 mL of 50 mM potassium phosphate buffer, pH 7.0) were mixed by vortexing for 1 min. To extract the lipid, 200 μL of HPLC-grade hexane was added and the suspension was mixed for an additional 1 min. Phases were separated by centrifugation at 550*g* for 2 min, and the hexane layer was withdrawn and evaporated under nitrogen. The residue was then redissolved in 50 μL of filtered HPLC-grade methanol by mixing, and 20 μL of the mixture was injected into an HPLC. The HPLC column was 3.9 mm × 30 cm stainless steel packed with micro Bondapak C-18. 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.

Liver lipid peroxidation was measured by assaying thiobarbituric acid reactive substances (TBARS) using a modification of the procedure described by Fraga et al. (1988). 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 min, and TBARS was 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 (Tokyo, Japan). The values were expressed in nmol/g liver. A malondialdehyde standard curve was also prepared, using 1,1,3,3,-tetramethoxypropane.

Hepatic Antioxidant Enzyme Activity Assays. Hepatic cytosolic and microsomal fractions of rats were prepared by differential centrifugation (Huang et al., 1988). Hepatic cytosolic GSH peroxidase activity was determined spectrophotometrically with a coupled procedure using H_2O_2 as the substrate (Lawrence and Burk, 1976). Hepatic cytosolic GSH reductase activity was measured as described by Bellomo et al. (1987). Hepatic GST activity was determined by the method of Habig et al. (1974). 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 et al. (1951).

Hepatic GSH Status Assay. Frozen liver tissue was used to determine the GSH redox status. GSH and GSSG were determined by HPLC as described by Reed et al. (1980) with some modification (Lii and Huang, 1997).

Plasma PGE_2 Analysis. The plasma PGE_2 content was analyzed by radioimmunoassay (New England Nuclear Corp., Boston, MA) according to the manufacturer's instructions.

Hepatic Enzyme-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 PGST- and GGT- positive focus assays. PGST-positive foci were visualized by immunohistochemical methods as described by Hendrich et al. (1991). Rabbit anti-PGST antiserum was kindly provided by Dr. Hendrich (Iowa State University, IA). PGST-positive foci were detected with a Vectastain ABC/peroxidase immunoassay kit (Vector Laboratories, Burlingame, CA). For color development, aminoethylcarbazole (AEC kit, Vector Laboratories, Burlingame, CA) was used as the substrate for peroxidase.

GGT-positive foci assays were performed according to the method described by Rutenburg et al. (1969). Briefly, the 10-μm liver sections were air-dried, submersed in freshly prepared γ-glutamyl-4-methoxy-2-naphthylamine solution for 15 min at room temperature, washed in 0.85% saline solution for 2 min, and stabilized with 0.1 M $CuSO_4$ for 2 min, washed in deionized water, and air-dried. Finally, we added glycerol gelatin and put the sections under glass covers. The sizes and numbers of GGT- and PGST-positive foci were quantified under a microscope with Leica Q500MC software (Germany). The foci were recognized only if their foci diameter was larger than 0.25 mm.

Table 3. Food Intake, Body Weight Gain, Liver Weight, Relative Liver Weight, Spleen Weight, and Relative Spleen Weight of Rats Treated with DEN/PB and Fed Fish Oil or Corn Oil for 24 Weeks[a]

	fish oil[b,c]	corn oil[b,c]
food intake (g)	3067 ± 163	3261 ± 248
body weight gain (g)	274 ± 35	271 ± 41
liver weight (g)	14.4 ± 1.6*	11.1 ± 1.6
liver weight/body weight (%)	4.3 ± 0.3*	3.4 ± 0.4
spleen weight (g)	1.0 ± 0.2*	0.8 ± 0.2
spleen weight/body weight (%)	0.3 ± 0.1*	0.2 ± 0.1

[a] Female pups are initiated with DEN (15 mg/kg) at 24 h of age. There are 12 rats in each group. Food intake is measured twice weekly. [b] Values are means ± SD. [c] Groups not sharing a symbol (*) are significantly different ($p < 0.05$).

Table 4. Hepatic Phospholipid Fatty Acid Profile of Rats Treated with DEN/PB and Fed Fish Oil or Corn Oil for 24 Weeks[a]

fatty acid	fish oil (%)[b,c]	corn oil (%)[b,c]
16:0	11.9 ± 1.5	10.3 ± 1.5
16:1	1.5 ± 0.3	1.3 ± 0.4
18:0	33.1 ± 1.5	36.3 ± 1.9*
18:1	1.8 ± 0.3	2.0 ± 0.3
18:2n-6	3.5 ± 0.7	5.4 ± 1.0*
20:0	8.7 ± 1.2	8.9 ± 2.4
18:3n-3	3.7 ± 0.9	3.5 ± 1.6
20:4n-6	17.1 ± 1.4	27.8 ± 3.3*
20:5n-3	4.7 ± 2.1*	ND
22:6	14.0 ± 1.8*	4.6 ± 1.0

[a] Female pups are initiated with DEN (15 mg/kg) at 24 h of age. There are 12 rats in each group. Food intake is measured twice weekly. [b] Values are means ± SD. [c] Groups not sharing a symbol (*) are significantly different ($p < 0.05$).

Statistical Analysis. All analyses were conducted in duplicate for each sample. Data were analyzed by using analysis of variance (ANOVA) (SAS Institute, Cary, NC). Student's t-test was used to evaluate the significance between means of treatment. Pearson correlation coefficients were also calculated. A value of $p < 0.05$ was taken to be statistically significant.

RESULTS

Food intake and body weight gain of rats fed fish oil or corn oil were not significantly different. Liver weight and relative liver weight were significantly greater in rats fed fish oil than in rats fed corn oil ($p < 0.05$) (Table 3). Spleen weight and relative spleen weight were significantly greater, as well, in rats fed fish oil than in rats fed corn oil ($p < 0.05$) (Table 3).

The hepatic phospholipid fatty-acid profile reflected the source of dietary lipids. Hepatic phospholipid 16:0, 16:1, 18:1, 20:0, and 18:3 n-3 were not significantly different in rats fed either fish oil or corn oil (Table 4). However, rats fed fish oil had significantly greater hepatic phospholipid 20:5 n-3 and 22:6 n-3 than rats fed corn oil ($p < 0.05$). In contrast, rats fed corn oil had significantly greater hepatic phospholipid 18:0, 18:2 n-6 and 20:4 n-6 than rats fed fish oil ($p < 0.05$) (Table 4).

The liver vitamin E content of rats was significantly affected by the source of dietary lipids. Rats fed fish oil had significantly lower liver vitamin E content than rats fed corn oil; liver lipid peroxidation was significantly greater in rats fed fish oil than in rats fed corn oil ($p < 0.05$) (Table 5). Liver GSH content was not significantly different in rats fed either fish oil or corn oil, but liver GSH/(GSH + GSSG) ratio, which reflects the redox

Table 5. Liver Vitamin E Content, Lipid Peroxidation, GSH Status, GSH Reductase, GSH Peroxidase, GST Activities, and Plasma PGE_2 Level of Rats Treated with DEN/PB and Fed Fish Oil or Corn Oil for 24 Weeks[a]

	fish oil[b,c]	corn oil[b,c]
vitamin E (μg/g)	7.7 ± 2.3	17.0 ± 5.9*
TBARS (nmol/g)	199.5 ± 10.8*	51.0 ± 12.1
GSH (nmol/mg protein)	23.8 ± 2.8	20.3 ± 4.5
GSH/(GSH + GSSG)	0.93 ± 0.01*	0.88 ± 0.02
GSH reductase (nmol/min/mg protein)	75.8 ± 6.9*	59.2 ± 6.8
GSH peroxidase (nmol/min/mg protein)	832 ± 80	843 ± 180
GST (nmol/min/mg protein)	3504 ± 642*	2607 ± 476
PGE_2 (ng/mL)	4.9 ± 1.1	7.3 ± 1.4*

[a] Female pups are initiated with DEN (15 mg/kg) at 24 h of age. There are 12 rats in each group. Food intake is measured twice weekly. [b] Values are means ± SD. [c] Groups not sharing a symbol (*) are significantly different ($p < 0.05$).

Table 6. Hepatic GGT- and PGST-Positive Foci of Rats Treated with DEN/PB and Fed Fish Oil or Corn Oil for 24 Weeks[a]

	fish oil[b,c]	corn oil[b,c]
GGT-positive foci		
foci area/tissue area (%)	0.14 ± 0.14	0.75 ± 0.52*
foci no./cm^2	1.5 ± 0.7	7.3 ± 3.1*
foci no./cm^3	21.7 ±13.8	122.7 ± 47.4*
PGST-positive foci		
foci area/tissue area (%)	0.58 ± 0.64	1.11 ± 1.16
foci no./cm^2	2.6 ± 2.6	8.7 ± 5.8*
foci no./cm^3	17.8 ± 12.9	141.4 ± 86.9*

[a] Female pups are initiated with DEN (15 mg/kg) at 24 h of age. There are 12 rats in each group. Food intake is measured twice weekly. [b] Values are means ± SD. [c] Groups not sharing a symbol (*) are significantly different ($p < 0.05$).

status of tissues, was significantly greater in rats fed fish oil than in rats fed corn oil ($p < 0.05$) (Table 5). Liver GSH reductase and GST activities were significantly greater in rats fed fish oil than in rats fed corn oil ($p < 0.05$) (Table 5). Dietary lipid source had no effect on hepatic GSH peroxidase activity. However, plasma PGE_2 level was significantly lower in rats fed fish oil than in rats fed corn oil ($p < 0.05$) (Table 5).

Dietary lipid source had a significant effect on hepatic enzyme-altered foci formation. Rats fed fish oil developed significantly fewer GGT-positive foci than rats fed corn oil ($p < 0.05$) (Table 6). The parameters of hepatic enzyme-altered foci measured included foci area/tissue area (%) and foci number (no./cm^2 or cm^3). Dietary lipid source had no effect on the PGST-positive foci area as a percentage of tissue area, however, rats fed fish oil had a significantly lower PGST-positive foci number (no./cm^2 or cm^3) than rats fed corn oil ($p < 0.05$) (Table 6).

The correlation coefficients between GGT- and PGST-positive foci number and plasma PGE_2, liver vitamin E, lipid peroxidation, GSH/(GSH + GSSG) ratio, GSH reductase, and GST activities were calculated. These results are shown in Table 7.

DISCUSSION

In the present study, we did not include the groups of rats without PB treatment. The reason was that in our previous study we found the high-fish-oil diet can inhibit GGT-positive foci formation compared to the high-corn-oil diet only in the presence of both DEN and PB (Chen et al., 1997a).

In this study, dietary lipid source had no effect on the growth of rats (Table 3). However, rats fed fish oil had significantly greater liver weight, relative liver weight, spleen weight, and relative spleen weight (Table 3). Significantly greater liver weights and relative liver weights of rats fed fish oil compared with those of rats fed corn oil were also found in our previous study. This led us to suggest that fish oil can exert a stimulatory effect on hepatic microsomal enzymes (Chen et al., 1996). In another previous study, we found that fish oil significantly increased hepatic microsomal cytochrome P-450 2B1 and 2E1 activities in comparison with those of corn oil (Chen et al., 1997b). There is evidence that long-chain fatty-acids-caused hepatomegaly partly accounted by their PPAR ligand property, and subsequently leads to increase in cytochrome P450 4A activity. These evidences suggest that the induction of peroxisome, mitochondria, and endoplasmic reticulum proliferation partly accounts for the enlargement of liver (Reddy et al., 1996; Gibson and Lake, 1993). Taken together, these results suggest that fish oil's effect on hepatic enzyme-altered foci may occur through its modulation of the hepatic detoxification system and lead to reduced promotion effect of PB. The main metabolic pathway of PB is hydroxylation and subsequent glucuronidation, and both the hydroxylate and glucuronidate forms of PB are inactive metabolites (Battino et al., 1995). In the present study, rats fed fish oil had significantly greater spleen weight and relative spleen weight compared to those of rats fed corn oil ($p < 0.05$). This result is in agreement with the finding of Blok et al. (1996) who showed that this increase was largely due to macrophage accumulation in the spleen.

In the present study, dietary lipid significantly affected the hepatic phospholipid fatty-acid profile of rats (Table 4). These results are consistent with those of our previous studies (Chen et al., 1997a,b). Changes in membrane composition and inhibition of intercellular communication by PB have been suggested to be responsible for its promoter function (Williams, 1981). The possibility that the effect of dietary lipid on hepatic enzyme-altered foci is through its action on membrane composition cannot be excluded. Levels and activities of liver antioxidants, antioxidant enzymes, lipid peroxidation, and plasma PGE_2 were significantly affected

Table 7. Correlation Coefficients between Foci Number and Plasma PGE_2, Liver Vitamin E, Lipid Peroxidation, GSH/(GSH + GSSG) Ratio, GSH Reductase, and GST Activities of Rats Treated with DEN/PB and Fed Fish Oil or Corn Oil for 24 Weeks

	PGE_2		Vitamin E		TBARS		GSH/(GSH + GSSG)		GSH reductase		GST	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
GGT-positive												
foci no./cm^2	0.66	0.01	0.57	0.04	−0.80	0.0006	−0.61	0.05	−0.75	0.002	−0.65	0.01
foci no./cm^3	0.66	0.01	0.49	0.09	−0.82	0.0003	−0.62	0.04	−0.75	0.002	−0.65	0.01
PGST-positive												
foci no./cm^2	0.56	0.02	0.62	0.007	−0.58	0.01	−0.40	0.14	−0.53	0.02	−0.44	0.07
foci no./cm^3	0.64	0.004	0.72	0.001	−0.72	0.0008	−0.53	0.04	−0.65	0.004	−0.52	0.03

by dietary lipids (Table 5). Rats fed fish oil had significantly lower hepatic vitamin E content than rats fed corn oil ($p < 0.05$). In contrast, liver lipid peroxidation (TBARS) was significantly greater in rats fed fish oil than in rats fed corn oil. These results are in agreement with those of Chen et al. (1997a) and Cho and Choi (1994), and suggest that the more double bonds in fish oil compared to corn oil *causes* the rats to consume more vitamin E. Also, the greater lipid peroxidation potential of fish oil compared to that of corn oil results from the higher number of double bonds in fish oil. In this study, rats fed fish oil had greater hepatic GSH content than rats fed corn oil, however, the difference was not significant. In addition, the hepatic GSH/total GSH ratio was significantly greater in rats fed fish oil than in rats fed corn oil ($p < 0.05$). Both of these observations may have been due to the significantly greater hepatic GSH reductase activity in rats fed fish oil than in rats fed corn oil ($p < 0.05$). GSH reductase is responsible for the conversion of oxidized GSH to reduced GSH. In this study, rats fed fish oil also had significantly greater hepatic GST activity than rats fed corn oil ($p < 0.05$). These increases in the activities of antioxidant enzymes (e.g., GSH reductase and GST) may imply that fish oil is an oxidative stress-causing factor (decreased hepatic vitamin E content and increased lipid peroxidation). Rats fed fish oil had significantly lower hepatic PGE_2 levels than rats fed corn oil ($p < 0.05$). This was *probably* due to the decreased supply of the dietary precursor (n-6 PUFA) for PGE_2 synthesis in the fish oil group. The data of Tables 4 and 5 were obtained from whole liver homogenate and we thought these changes occur in the foci as well.

Except for the PGST-positive foci measured as foci area/tissue area (%), all the other parameters measured were significantly greater in rats fed corn oil than in rats fed fish oil (Table 6). These results support previous observations in animals that intake of n-6 PUFA promotes the development of tumors in colon, mammary gland, pancreas, and liver compared to intake of n-3 PUFA (Reddy and Maruyama, 1986; Jurkowski and Cave, 1985; O'Conner et al., 1989; Chen et al., 1997a; Sugie et al., 1995; Rahman et al., 1999). In other words, n-3 PUFA can inhibit tumor promotion compared to n-6 PUFA. To determine the possible mechanisms involved in fish oil inhibition of hepatic enzyme-altered foci, we analyzed the Pearson's correlation coefficients between hepatic enzyme-altered foci, plasma PGE_2 level, hepatic vitamin E content, lipid peroxidation, GSH/(GSH +GSSG) ratio, GSH reductase and GST activities, respectively (Table 7). Most of the correlation coefficients were found to be statistically significant. The positive correlation found between hepatic enzyme-altered foci and PGE_2 supports the promotion role of PG in tumor development in various organs in animals, and inhibition of PG synthesis can suppress experimental chemical carcinogenesis (Mernett, 1992; McCormick et al., 1985; Takahashi et al., 1990; Shibata et al., 1993; Denda et al., 1989; Denda et al., 1994). The positive correlation found between hepatic enzyme-altered foci and vitamin E, and the negative correlation between hepatic enzyme-altered foci and lipid peroxidation and GSH-related antioxidant capacity (GSH/(GSH + GSSG), GSH reductase, and GST activities), implies that increased oxidative stress can reduce the susceptibility of animals to tumor promotion. The increases in GSH-related antioxidant capacity in the fish oil group compared to those in corn oil group in this study may have been due to the oxidative stress exerted by the fish oil and then enhanced the GSH-related antioxidant capacity to defend against the oxidative stress. Dormandy and Wickens (1988) and Cerutti et al. (1988) proposed that the increased antioxidant capacity and decreased lipid peroxidation conditions favored the neoplastic growth of initiated cell. The liver weight and relative liver weight were significantly greater in rats fed fish oil than in rats fed corn oil (Table 3). In addition to the stimulatory effect on hepatic microsomal enzymes by fish oil, the other possibilities may include the greater number of Kupffer cells found in nontransformed liver than in hepatic enzyme-altered foci as suggested by Janossy et al. (1986). In this study, the fish oil group had significantly fewer hepatic enzyme-altered foci than the corn oil group.

In summary, fish oil significantly inhibited hepatic enzyme-altered foci formation compared to corn oil. The possible mechanisms involved in this process include stimulation of the hepatic detoxification system, changes in membrane composition, inhibition of PGE_2 synthesis, enhancement of GSH-related antioxidant capacity, and increased lipid peroxidation by fish oil in the presence of both DEN and PB.

ABBREVIATIONS USED

DEN, diethylnitrosamine; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GGT, γ-glutamyl transpeptidase; PB, phenobarbital; PG, prostaglandins; PGST, placental form of glutathione S-transferase; PUFA, polyunsaturated fatty acids; TBARS, thiobarbituric acid reactive substances.

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