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Inhibitory Effect of Dietary Perilla Oil Rich in the n-3 Polyunsaturated Fatty Acid α-Linolenic Acid on Colon Carcinogenesis in Rats

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The inhibitory effect of dietary perilla oil rich in the n-3 polyunsaturated fatty acid α-linolenic acid against colon carcinogenesis was investigated in rats. Four groups of 26 F344 rats each received an intrarectal dose of 2 mg of N-methyl-N-nitrosourea 3 times a week for 2 weeks, and received a diet containing 12% perilla oil, 6% or 12% safflower oil (rich in the n-6 polyunsaturated fatty acid linoleic acid), or 12% palm oil (rich in saturated and monounsaturated fatty acids). At week 35, the incidence of colon cancer was significantly lower in perilla oil-fed rats than in other dietary groups; 19% vs. 46%, 56% and 58%. When examined at week 10, the concentration of fecal bile acids, known to be tumor promoters, was not significantly different among the dietary groups, and the intrarectal deoxycholic acid-induced colonic mucosal ornithine decarboxylase activity, a marker of tumor promotion, was significantly lower in perilla oil-fed group than in other groups. The serum and colonic mucosal fatty acid compositions and the blood plasma prostaglandin E_2 level directly reflected the fatty acid composition of each dietary fat. The results suggest that the anti-tumor-promoting effect of dietary perilla oil was a result of a decreased sensitivity of colonic mucosa to tumor promoters arising from the altered fatty acid composition in membrane phospholipid of colonic epithelial cells, and was not a consequence of a decrease of promoters such as bile acids.

Key words: Colon carcinogenesis — Perilla oil — α-Linolenic acid — Ornithine decarboxylase — Cancer prevention

Epidemiologic studies have demonstrated a strong association between high fat intake and an increased risk of colon cancer.[1,2] Studies on animal models have made clear that diets high in fats from either animal and vegetable sources enhance the development of chemical carcinogen-induced colon cancer.[1] However, some fats, coconut oil and olive oil, did not show such an effect in rats.[3] These fats are known to have compositions low in the n-6 family polyunsaturated fatty acid (PUFA)[5] linoleic acid (C18:2n-6), which is an essential fatty acid, and a precursor of arachidonic acid (C20:4n-6), prostaglandins and leukotrienes, although most vegetable oils have a composition high in linoleic acid. Furthermore, a high fish oil diet did not enhance, but rather inhibited colon cancer development in rats.[4-6] Fish oil has a unique fatty acid composition, namely it contains an appreciable amount of n-3 family PUFAs, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), and a small amount of n-6 PUFAs. Thus, the type or the fatty acid composition of fats seems to be a significant determining factor in colon carcinogenesis. In this respect, it is of interest that the mortality from colon cancer is low in Greece where olive oil is often used in cooking, and in Greenland Eskimos and Asians including Japanese people, who frequently eat marine fish.[1,7] However, the incidence of this cancer in Japan has been increasing in recent years, as dietary habits have become Westernized.[8] Another essential fatty acid, α-linolenic acid (C18:3n-3), is converted to EPA and DHA in the liver, and these n-3 PUFAs replace n-6 PUFAs in membrane phospholipid pools in various types of cells.[9] Those metabolic and biological properties of n-3 PUFAs prompted us to investigate the effect of dietary perilla oil (rich in α-linolenic acid) on colon carcinogenesis.

In the present investigation, 12% perilla oil-containing diet was given to colonic carcinogen-treated rats. They had 25% of their total caloric intake from the fat in the diet. This is similar to the ratio of caloric intake from fat in Japanese people. Control rats were fed diets containing safflower oil (rich in linoleic acid), or palm oil (rich in saturated and monounsaturated fatty acids). Perilla oil

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[5] Abbreviations used are: PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ODC, ornithine decarboxylase; MNU, N-methyl-N-nitrosourea; PGE_2, prostaglandin E_2.

diet inhibited colon cancer development along with changes of serum and colonic mucosal fatty acid composition, blood plasma prostaglandin E_2 (PGE_2) level and colonic mucosal ornithine decarboxylase (ODC) activity, but not fecal bile acids. The result indicated an anti-tumor-promoting effect of α-linolenic acid.

MATERIALS AND METHODS

Animals Female F344/NSlc rats (Shizuoka Laboratory Animal Center, Hamamatsu), 7 weeks of age at the start of the experiment, were used. They were housed, 5 rats each, in a plastic cage with sterilized wood-chip bedding in a specific-pathogen-free animal room under constant conditions with a 12-h light-dark cycle, a temperature of 22 ± 1°C, a relative humidity of 50 ± 10%, and free access to drinking water and diet.

Chemicals and dietary fats The carcinogen N-methyl-N-nitrosourea (MNU, Nakarai Chemicals Co., Kyoto) was dissolved to make a 0.4% aqueous solution at pH 4.7 immediately before use. Three dietary fats tested, safflower oil, perilla oil and liquid palm oil, were refined and 300 ppm α-tocopherol and 200 ppm ascorbic acid were added at Tsukuba Research Laboratories, Nippon Oil and Fats Co., Tsukuba. Other antioxidants were not used. The fatty acid compositions of the fats are listed in Table I.

Diets Four experimental diets in pelletted form were prepared once a month by adding one of the dietary fats to basal laboratory diet MB-1 at the expense of starch at Funabashi Farm Co., Funabashi. The composition of MB-1 diet is 24% protein, 54% starch, 3% fat, 4% fiber, 9% water and 6% others including mineral mixture and vitamin mixture. They were supplemented with 6% safflower oil (sf diet), 12% safflower oil (SF diet), 12% perilla oil (PR diet) or 12% palm oil (PL diet) by weight. Thus, the diets contained 9% or 15% fat in total. After preparation, the diets were sealed in 1 kg air-tight plastic bags, transported in a refrigerated truck, and then stored in a deep-freezer at -60°C until use. The diets in the container in the animal cage were shaded from light. Peroxidase value of fats in SF and PR diets was confirmed to be below 3 before use and below 10 after one day in the container in the cage.

Table I. Fatty Acid Composition of Dietary Fats[a)]

Fatty acids	Safflower oil	Perilla oil	Palm oil
C14:0	—	—	1%
C16:0	8%	7%	36
C18:0	3	2	5
C18:1	17	19	40
C18:2n-6	69	14	17
C18:3n-3	2	58	1
C20:1	1	—	—

a) α-Tocopherol 300 ppm and ascorbic acid 200 ppm were added.

Animal treatments Four groups of rats consisting of 26 rats each were placed on sf, SF, PR or PL diet at week 1 until the termination of the experiment. The diet containers were changed daily, and the weight of diet consumed was recorded. The body weight was measured once a week. All the rats received an intrarectal instillation of 0.5 ml of 0.4% MNU aqueous solution 3 times a week at weeks 1 and 2 by the procedure described previously.[10] Briefly, a metal feeding tube 8 cm long was inserted about two-thirds of the way into the large bowel lumen through the anal orifice, and the solution was infused. These rats served for the investigation of colon cancer development at week 35 and for the analysis of fecal bile acids at week 10.

Other groups of 6 rats each without carcinogen treatment were fed sf, SF, PR or PL diet, and were used for analyses of serum fatty acids, colonic mucosal fatty acids, blood plasma PGE_2 and colonic mucosal ODC activity at week 10.

Colon tumor examination The experiment was terminated at week 35, and all the rats were killed. At autopsy, the large bowel was cut open along its length and inspected grossly. The location, size and shape of colonic tumors were recorded. All the tumors and grossly abnormal tissues or organs were histologically examined after standard processing, sectioning and staining with hematoxylin and eosin.

Fecal bile acid assay Feces from 5 rats each on sf, SF, PR or PL diet were collected for 3 days at week 10, and stored at -80°C until bile acid measurement. Freeze-dried feces were processed as described previously.[11] Briefly, a ground sample (100 mg) was refluxed in organic solvents, and bile acids were separated on Lipidex-DEAP (Packard Instrument, Downers Grove, III) into unconjugated and conjugated fractions. Conjugated fractions were hydrolyzed. The resultant products were analyzed in duplicate by capillary gas chromatography and mass spectrometry. The results were expressed as the average value of μmol per g dry feces.

Serum and colonic mucosal fatty acid assay Blood from 6 rats each on SF, PR or PL diet for 10 weeks was collected from the abdominal aorta after laparotomy under intraperitoneal Nembutal anesthesia at 40 mg/kg body weight, and was centrifuged. Then, the large bowel was excised, cut open lengthwise and rinsed with cold 0.9% NaCl solution to remove fecal debris. The mucosa of the distal large bowel was scraped with a blunt steel plate, and immediately frozen. Total lipid in the serum and the mucosa was extracted by the method of Bligh and Dyer.[12] Fatty acids were trans-esterified with 5%

HCl in methanol, and analyzed by gas-liquid chromatograph (Shimadzu GC-7AG, Shimadzu Seisakusho, Kyoto) using a flame ionizing detector. The column temperature was 220°C and the injection temperature was 270°C. Peak areas were measured using an integrator (Shimadzu C-RIA). The composition of individual fatty acids was expressed as percentage of total area of all fatty acids.

Plasma PGE_2 assay Blood from 6 rats each obtained as described was collected in a test tube containing 0.1 ml of solution with 7×7^{-7} *M* indomethacin, 7×10^{-5} *M* EDTA and 100 U of aprotinin, and was centrifuged immediately. The plasma was acidified to pH 3.5 to 4.0 by addition of 0.05 ml of 2 *N* HCl per ml of plasma to denature protein, and was extracted on a Bond-Elut C-18 column (Analytichem International, Harber, CA). The PGs extract obtained was assayed for PGE_2 content using PGE_2-^{125}I RIA kit (Du Pont/NEN Research Products, Boston, MA). The assay was done in duplicate, and the average value was expressed as pg/ml.

Assay of colonic mucosal ODC activity Four groups of 6 rats each on sf, SF, PR or PL diet for 10 weeks received an intrarectal instillation of 1 ml of 0.9% NaCl solution containing 12 μmol of sodium deoxycholate (Nakarai Chemicals Co.) under intraperitoneal Nembutal anesthesia. Four hours after the dose, the rats were killed by cervical dislocation. The mucosa of the distal large bowel, which had been exposed to the instilled solution, was obtained as described. One hundred mg of the mucosa was homogenized in 4 ml of 50 m*M* sodium phosphate buffer (pH 7.2) containing 0.1 m*M* pyridoxal phosphate and 0.1 m*M* EDTA. The supernatant fraction obtained after centrifugation at 30,000*g* for 30 min at 2°C was used as the enzyme extract. The enzyme activity was determined in duplicate by measuring the release of $^{14}CO_2$ from DL-[1-C^{14}]ornithine hydrochloride (56 mCi/mmol, Amersham Inst., Buckinghamshire, England) as a substrate, as described previously.[13] Protein content of the mucosal extract was measured by using a Bio-Rad Assay kit (Bio-Rad Lab., Richmond, CA). The average value was expressed as pmol of CO_2 liberated in 60 min per mg of protein.

Statistical analysis One rat of the SF group died of acute pneumonia at week 20, and was excluded from the study. Differences between dietary groups were tested for statistical significance by using the χ^2 test and Student's *t* test. The results were considered statistically significant if the *P* value was 0.05 or less.

RESULTS

Body weight gain and food consumption Body weight gain was significantly smaller (6–10%) in the sf group compared to the SF, PR and PL groups, but was essentially the same among the latter 3 groups, namely 141±4 (mean±SD) g vs. 149±6 g, 153±7 g and 156±7 g (Table II). The total amounts of diet consumed were roughly the same in the SF, PR and PL groups, but the amount was larger (8–10%) in the sf group compared to the other 3 groups (Table II). The values of total caloric intake, calculated from the amounts of consumed diet (369 kcal/100 g of sf diet, and 399 kcal/100 g of SF, PR and PL diets), showed no significant difference among the groups.

Colon tumor development The incidence and the number of MNU-induced colon tumors in rats autopsied at week

Table II. Body Weight and Consumed Diet of F344 Rats Fed Diets Supplemented with Different Types of Fats

Treatment group[a]	at week							
	0	5	10	15	20	25	30	35
	Body weight (g)							
sf	119±2[b]	182±3	205±4	217±3	229±4	240±4	251±4	260±3
SF	123±1	181±1	204±4	218±5	234±4	247±6	258±7	272±6
PR	120±3	177±1	199±2	217±3	235±3	248±5	261±6	273±6
PL	121±2	186±3	205±2	231±8	241±4	253±4	266±5	277±8
	Consumed diet (g/day/rat)							
sf	14.3±1.6[b]	10.6±0.5	10.2±0.9	9.0±0.6	9.4±0.5	9.6±0.4	9.6±0.7	10.0±0.5
SF	13.0±1.6	10.1±0.3	8.5±0.6	8.3±0.5	8.6±0.5	9.0±0.6	8.6±0.6	9.7±0.5
PR	12.9±1.7	9.9±0.2	8.3±0.6	8.5±0.4	8.7±0.7	8.5±0.7	8.7±0.7	9.4±0.3
PL	13.5±1.6	9.8±1.0	8.6±0.5	8.0±0.5	9.0±0.3	9.2±0.5	8.9±0.8	9.4±0.5

a) All 26 rats in each group received an intrarectal dose of 2 mg of MNU 3 times a week for 2 weeks, and were fed with diet containing 6% safflower oil (sf group), 12% safflower oil (SF group), 12% perilla oil (PR group) or 12% palm oil (PL group). The experiment was terminated at week 35.
b) Mean±SD.

35 are summarized in Table III. The incidence in the PR group was significantly lower than those in the other groups (sf, SF and PL), namely 19% vs. 46%, 56% and 58%, respectively. The incidence in the sf group was lower than that in the SF group, but the difference was not significant. Also, the mean number of tumors per rat was significantly smaller in the PR group than in the other groups. The tumor-bearing rats had one tumor each in the PR group, but 1 to 3 tumors in other groups. It was noted that the tumor size in the PR group fed with 12% perilla oil diet, a higher fat diet, was significantly smaller than that in the sf group fed with 6% safflower oil diet, a lower fat diet. The data clearly demonstrated that 12% perilla oil diet did not enhance, but rather suppressed, the colon tumor development.

All the tumors were located in the distal half of the large bowel, which had been bathed with instilled MNU solution, and they were plaque-shaped or polyoid. Histologically, all the tumors were well-differentiated adenocarcinomas. Most of the tumors were small with minimal extension and no metastasis to lymph nodes or other organs, because the experiment was terminated early, at week 35. All 5 tumors in the PR group and all 20 tumors in the SF group were 6 mm or smaller in diameter, while 1 of 16 tumors in the sf group and 7 of 23 tumors in the PL group were larger, ranging from 7 to 11 mm. All 5 tumors in the PR group were located within the mucosa, while 5 tumors in the sf group, 2 tumors in the SF group and 6 tumors in the PL group had invaded the submucosa or the proper muscle. Thus, the tumors in the PR group appeared to be much less extensive, in contrast to the tumors in the PL group. There were no other pathologic findings in the gastrointestinal tract or other organs in any of the groups of rats.

Table III. MNU-induced Colon Cancer in F344 Rats Fed Diets Supplemented with Different Types of Fats

Treatment group[a]	No. of rats with tumors	No. of tumors per rat	No. of tumors per tumor-bearing rat
sf (26)[b]	12 (46%)	0.6±0.1[c]	1.3±0.1
SF (25)	14 (56%)	0.8±0.2	1.4±0.1
PR (26)	5 (19%)[d]	0.2±0.1[d]	1.0
PL (26)	15 (58%)	0.9±0.2	1.5±0.2

a) See Table II or text.
b) Effective number of rats.
c) Mean±SEM.
d) Significantly different from other groups: $P<0.05$ or 0.01.

Fecal bile acids The total amount of fecal bile acids at week 10 was similar among the SF, PR and PL groups (Table IV). The concentration of total bile acids was significantly lower in the PL group than the SF and PR groups. The concentrations of lithocholic acid and deoxycholic acid in the PR group were significantly lower and higher, respectively, compared to the SF and PL groups. Thus, the concentration of total secondary bile acids (lithocholic acid plus deoxycholic acid, both of which are potent tumor promoters in colon carcinogenesis) was not significantly different among groups, 2.50±0.16 (mean±SEM) μmol/g dry feces in the SF group, 2.89±0.20 μmol/g dry feces in the PR group and 2.30±0.21 μmol/g dry feces in the PL group. The data appeared to indicate that bile acid levels did not account for the difference of colon tumor development among groups of rats fed with different types of fats.

Serum and colonic mucosal fatty acids The fatty acid composition in serum and colonic mucosal lipids at week 10 was markedly high in saturated and monounsaturated fatty acids in the PL group, in n-6 PUFAs in the SF group and in n-3 PUFAs in the PR group (Tables V and VI). It was noted that the ratio of n-3 PUFAs to n-6 PUFAs was very low in the SF and PL groups compared to the PR group, namely 0.1 and 0.3 vs. 1.0 in the serum, and 0 (calculated) and 0.08 vs. 1.1 in the mucosa. These differences may be directly attributed to the respective

Table IV. Fecal Bile Acids in F344 Rats Fed Diets Supplemented with Different Types of Fats

Treatment group[a]	Concentration of bile acids (μmol/g dry feces)						Total amount of bile acids (μmol/day/rat)
	Litho-cholic acid	Deoxy-cholic acid	Hyodeoxy-cholic acid	β-Muri-cholic acid	Others	Total bile acids	
SF	0.73±0.03[b]	1.77±0.13	3.87±0.19	0.57±0.03	3.24±0.17	10.17±0.50	13.50±1.49
PR	0.57±0.02[c]	2.32±0.20[d]	3.86±0.14	0.66±0.04	3.65±0.15	11.07±0.42	14.75±0.49
PL	0.63±0.04	1.67±0.17	3.09±0.25[d]	0.49±0.09	2.62±0.20[d]	8.52±0.72[e]	13.70±0.66

a) See Table II or text. Feces from 5 rats each were collected for 3 days at week 10.
b) Mean±SEM.
c) Significantly different from the SF group: $P<0.01$.
d) Significantly different from the other groups: $P<0.05$.
e) Significantly different from the PR group: $P<0.02$.

Table V. Percent Composition of Serum Fatty Acids in F344 Rats Fed Diets Supplemented with Different Types of Fats

Treatment group[a)]	Saturated FAs		Mono-UFAs		n-6 PUFAs		n-3 PUFAs			Others
	C16 : 0	C18 : 0	C16 : 1	C18 : 1	C18 : 2	C20 : 4	C18 : 3	C20 : 5	C22 : 6	
SF	$18.4\pm0.4^{b)A}$	$11.5\pm0.7^{A,B}$	1.3 ± 0.1^{A}	8.6 ± 0.4^{A}	33.9 ± 1.3^{A}	15.6 ± 1.1^{A}	0.3 ± 0.03^{A}	0.9 ± 0.1^{A}	$4.2\pm0.2^{A,B}$	5.3 ± 0.3^{A}
PR	15.2 ± 0.3^{A}	13.1 ± 0.5^{A}	1.3 ± 0.1^{A}	11.2 ± 0.4^{B}	18.2 ± 0.2^{B}	7.7 ± 0.4^{B}	12.7 ± 0.6^{B}	11.1 ± 0.6^{B}	3.7 ± 0.3^{A}	5.8 ± 0.3^{A}
PL	26.8 ± 0.9^{B}	9.7 ± 0.7^{B}	2.5 ± 0.1^{B}	25.5 ± 1.2^{C}	15.2 ± 0.3^{C}	8.1 ± 1.0^{B}	0.4 ± 0.03^{A}	1.9 ± 0.1^{C}	4.7 ± 0.2^{B}	5.2 ± 0.3^{A}

a) Six rats each without MNU treatment were fed diets containing 12% safflower oil (SF group), 12% perilla oil (PR group) or 12% palm oil (PL group) for 10 weeks, then blood was collected.
b) Mean±SEM.
A, B and C. Means in the same column that do not share a common superscript letter are significantly different: $P<0.05$ or less.

Table VI. Percent Composition of Colonic Mucosal Fatty Acids in F344 Rats Fed Diets Supplemented with Different Types of Fats

Treatment group[a)]	Saturated FAs		Mono-UFAs		n-6 PUFAs		n-3 PUFAs			Others
	C16 : 0	C18 : 0	C16 : 1	C18 : 1	C18 : 2	C20 : 4	C18 : 3	C20 : 5	C22 : 6	
SF	$15.7\pm0.2^{b)A}$	7.7 ± 0.5^{A}	0.7 ± 0.2^{A}	17.6 ± 0.2^{A}	47.8 ± 1.0^{A}	7.0 ± 0.9^{A}	0^{A}	0^{A}	0^{A}	3.5 ± 0.2^{A}
PR	15.7 ± 0.6^{A}	7.2 ± 0.5^{A}	1.7 ± 0.2^{B}	25.3 ± 0.7^{B}	20.0 ± 0.2^{B}	1.6 ± 0.5^{B}	19.7 ± 1.1^{B}	2.8 ± 0.4^{B}	1.8 ± 0.4^{B}	$5.0\pm1.1^{A,B}$
PL	24.0 ± 1.1^{B}	6.9 ± 0.4^{A}	2.2 ± 0.6^{B}	38.3 ± 0.5^{C}	16.5 ± 0.6^{C}	4.6 ± 0.3^{C}	0.5 ± 0.1^{C}	0.3 ± 0.2^{A}	1.0 ± 0.3^{B}	5.7 ± 0.6^{B}

a) See Table V or text. Colonic mucosa was collected after death.
b) Mean±SEM.
A, B and C. Means in the same column that do not share a common superscript letter are significantly different: $P<0.05$ or less.

dietary fats. A significant increase of arachidonic acid in the SF group and a significant increase of EPA in the PR group were observed, showing that ingested linoleic acid and α-linolenic acid were converted significantly to arachidonic acid and EPA, respectively, *in vivo*, because neither of these fatty acids was detected in the oils used.

Plasma PGE_2 Plasma PGE_2 levels of 6 rats in each group at week 10 displayed a wide range, 1.1–7.5 ng/ml in the SF group, 0.05–0.35 ng/ml in the PR group and 0.6–8.5 ng/ml in the PL group. The mean value of the PR group was significantly lower than those of the SF and PL groups, namely 0.17±0.06 (mean±SEM) ng/ml vs. 4.7±1.1 ng/ml and 2.3±1.4 ng/ml. The data may indicate lower PGE_2 production, but not increased degradation, in the perilla oil-fed rats, resulting from lower n-6 PUFAs in membrane phospholipid.

Colonic mucosa ODC activity Deoxycholic acid-induced colonic mucosal ODC activity of 6 rats in each group at week 10 was remarkably low in the PR group compared to the other groups, being in the ranges of 0.08–0.20 nmol CO_2/60 min/mg protein in the PR group, 0.13–0.47 nmol CO_2/60 min/mg protein in the sf group, 0.15–0.58 nmol CO_2/60 min/mg protein in the SF group and 0.15–0.32 nmol CO_2/60 min/mg protein in the PL group. The mean enzyme activity was significantly lower in the PR group than in the other groups, namely 0.12±0.02 (mean±SEM) nmol CO_2/60 min/mg protein vs. 0.28±0.05 nmol CO_2/60 min/mg protein in the sf group, 0.39±0.06 nmol CO_2/60 min/mg protein in the SF group and 0.23±0.02 nmol CO_2/60 min/mg protein in the PL group. The enzyme activity of the SF group was insignificantly and significantly higher than those of the sf and PL groups, respectively. The data indicate that dietary safflower oil was responsible for the higher level of the enzyme induction, but dietary perilla oil did not lead to enzyme induction in the colonic mucosa. Again, it seems that linoleic acid resulted in induction of a much higher level of the enzyme, but α-linolenic acid did not.

DISCUSSION

Epidemiologic studies have shown a positive correlation between high fat diets and colon cancer as well as breast cancer.[14] Even though the human studies could not by themselves establish a causative relationship between those cancers and dietary fats, the evidence from animal studies, which have clearly demonstrated a promoting effect of high fat diets on colon[1] and mammary gland[15] carcinogenesis, may support an important role of dietary fats. However, recent animal studies have in-

dicated that the type or fatty acid composition of fats is a significant determining factor. The present study showed that the diet with 12% perilla oil, which is rich in the n-3 PUFA α-linolenic acid, inhibited MNU-induced colon tumor development in rats, compared to the diets with 12% or 6% safflower oil, which is rich in the n-6 PUFA linoleic acid. It has also been found that 15% perilla oil diet reduced 1,2-dimethylhydrazine-induced colon tumors in rats.[16] It is noteworthy that a significantly lower tumor yield in rats fed high fat diet with perilla oil was observed, even when compared to rats fed a lower fat diet with safflower oil, in the present investigation. The results seem to suggest that dietary linoleic acid and α-linolenic acid are involving in promotion and antipromotion, respectively, of cōlon carcinogenesis.

The fatty acid composition of fats in the diets was directly reflected in the fatty acid compositions in the serum and the colonic mucosa. Thus, the high ratio of n-3 PUFAs to n-6 PUFAs as observed in perilla oil-fed rats may be a key factor determining the inhibition of colon carcinogenesis, in contrast to safflower oil-fed rats. Caloric intake was not different among the dietary groups. Again, the different types of fats may operate differently to modulate colon cancer development, i.e., enhancement by n-6 PUFAs and inhibition by n-3 PUFAs. A selective incorporation of n-3 PUFAs and a competitive reduction of n-6 PUFAs in membrane phospholipid pools of cells in various organs in rats fed with n-3 PUFAs were demonstrated.[5, 17–19] Changes in the balance between n-3 and n-6 PUFAs in membrane phospholipid may affect the physiologic properties of the membrane and the membrane-bound enzymes, which regulate the sensitivity of cells to carcinogenic stimuli and tumor growth.[20, 21] Furthermore, it was reported that EPA and DHA inhibit the production of 2 series of eicosanoids, products derived from arachidonic acid.[22, 23] These eicosanoids, particularly PGE_2, are involved in the promotion phase of colon carcinogenesis,[24] because prostaglandin synthesis inhibitors such as indomethacin inhibit the tumor development.[25–27] It was confirmed in the present experiment that PGE_2 was very low in the blood of perilla oil-fed rats, although it was not measured in the colonic mucosa. However, direct evidence of a causal relationship between prostaglandins and membrane phospholipids, and colon carcinogenesis remains to be obtained.

Bile acids, particularly secondary bile acids, exert a tumor-promoting effect on colon carcinogenesis in animal models. Bile acids instilled into the colon lumen or fed at high dose in the diet enhanced colon tumor development in rats.[28–31] The treatments also caused hyperproliferation and unscheduled DNA synthesis of crypt cells, and an increased ODC activity in the colonic mucosa,[24, 32–34] which are biological markers of tumor promotion. High fat diets increase the excretion of bile acids, especially secondary bile acids, in the feces in rats as well as humans.[3, 35] The present investigation showed that the amount and the concentration of total bile acids and secondary bile acids in the feces were not different among the dietary groups. This indicates that the quality of dietary fats used did not influence bile acid metabolism, for which the same amounts of fats were consumed.

The induction of ODC, a first-step and rate-limiting enzyme of polyamine synthesis, is a useful biological parameter for tumor promotion. An increase of this enzyme activity in rat colonic mucosa by bile acids[24, 34] and in mouse skin by the phorbol ester TPA[36, 37] was associated with enhanced tumor induction in the colon and the skin, respectively. The diet high in linoleic acid-rich corn oil increased the colonic mucosal ODC activity and azoxymethane-induced colon tumor development.[38, 39] A significant decrease of the enzyme activity in perilla oil-fed rats was observed in the present study. This result may suggest that, as a whole, the inhibition of colon tumor development in perilla oil-fed rats was a consequence of reduced sensitivity of colonic epethelial cells to tumor-promoting stimuli such as bile acids. n-3 Fatty acids presumably modify the sensitivity of colonic epithelial cells to tumor promoters, perhaps through a membrane effect.

In conclusion, the present study indicated that the antitumorigenic effect of dietary perilla oil on colon carcinogenesis resulted from the reduced sensitivity of colonic epithelial cells to tumor promoters, following the change of the fatty acid composition in membrane phospholipid pools. However, the precise mechanisms of the inhibitory effect, and the required amounts of α-linolenic acid or perilla oil in total fat intake for preventing colon tumor development remain to be investigated. It is noteworthy that a decrease of azoxymethane-induced colon tumors and colonic mucosal ODC activity in rats fed diets with various levels of fish oil plus corn oil was reported.[19] High intake of n-3 PUFAs has several other pharmacologic properties; an antiinflammatory effect, altered lipoprotein metabolism, inhibition of atherosclerosis and retardation of tumor growth.[9, 21, 40] The levels of serum lipids measured in the present investigation were the highest in safflower oil-fed rats and the lowest in perilla oil-fed rats (the data are not shown). In addition to those benefits of α-linolenic acid, the peroxidation value of this fatty acid is different by a factor of 10 from that of EPA or DHA.[41] This chemical property is advantageous for dietary manipulation and pharmaceutical application of perilla oil as well as linseed oil rich in the n-3 PUFA α-linolenic acid, in contrast to the marine n-3 PUFAs, EPA and DHA.

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