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Effects of Dietary Fats and Fibers on Modulation of Biomarkers and Tumor Incidence in Rats during 1,2-Dimethylhydrazine-Induced Colon Carcinogenesis*

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ABSTRACT

This study investigated the effect of different dietary fats and fibers on colon tumor incidence and cell proliferation, the levels of eicosanoids and polyamines in colonic mucosa of DMH-treated rats. The experiment was conducted on male Sprague Dawley rats using a 2 × 3 factorial design with two fats (corn oil and DHA-rich fish oil) and two fibers (cellulose and pectin) and a fiber-free control. The rats were fed an experimental diet containing 15% (w/w) dietary fat and 6% (w/w) fiber for 25 weeks. Tumor incidence was lower in rats fed fish oil as opposed to corn oil. The levels of arachidonic acid (AA) and eicosanoids (PGE_2 and TXB_2) in normal colonic mucosa were significantly lower in rats fed fish oil and there was a concomitant increase of docosahexaenoic acid (DHA). The levels of eicosanoids and AA in tumor tissues were significantly higher than those of normal colonic mucosa. The level of polyamines in normal colonic mucosa was not affected by dietary fats but was significantly lower than that in tumor tissues. Dietary fiber did not have a significant effect on tumor incidence and the levels of AA, eicosanoids and polyamines. Overall, fish oil rich in DHA reduced cell proliferation and thus inhibited colon carcinogenesis through its effect on the distribution of AA and production of eicosanoids in normal colonic mucosa. However, its effect on colon carcinogenesis revealed a lack of consistency depending on the type of dietary fiber in diet.

KEY WORDS: colon cancer, cell proliferation, eicosanoids, polyamines, DHA, fiber.

INTRODUCTION

Many studies[1,2] demonstrated the beneficial effects of fish oil rich in n-3 polyunsaturated fatty acid (PUFA) in cancer treatment. High intake of EPA and DHA has been correlated with lower incidence of colon cancer. Conversely, fats high in n-6 PUFA appear to enhance tumorigenesis and tumor cell growth.[3] Explanatory theories have focused on the relative differences in the conversion of these n-6 and n-3 families of fatty acids into biologically active eicosanoids.[4] Since highly altered prostraglandin profiles are found in cancerous tissue, it is thought that eicosanoids may be involved in the etiology of this disease. A number of studies[5,6] have shown an increased level of AA in tumors of the colon. Although the role of eicosanoid in colon carcinogenesis is still unclear, colonic prostaglandin synthesis would involve the acute modulation of the proliferative activity of colonic epithelium.[6] Polyamines - putrescine, spermidine, and spermine - are ubiquitous compounds normally required for cell proliferation.[7] Several findings[7,8] have indicated that polyamine levels and ornithine decarboxylase (ODC) activity markedly increased not only in rapidly proliferating cells but also during neoplastic transformation.

Dietary fiber was reported to modify the rates of colonic cell proliferation by producing structural and functional changes in the intestinal epithelium.[9] A high fiber diet has been associated with a lower frequency of colon cancer.[9] But experimental study[10] of the effects of fiber on colon carcinogenesis have proved inconclusive. We investigated the effect of dietary fat and fiber on tumor incidence, cell proliferation, polyamines, eicosanoid levels and fatty acid composition of colon carcinogenesis.

METHODS AND MATERIALS

1. Experimental design

A total of 150 seven-week-old male Sprague Dawley rats (Animal care laboratory of Seoul National University) were randomly divided into 2 groups based on the type of fat, corn oil (CO) and DHA-rich fish oil (FO), and each group was further subdivided into 3 groups (fiber-free, pectin and cellulose). The rats were housed in cages and maintained in a temperature and humidity controlled animal facility with a daily photoperiod of 12h light and

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Table 1. Dietary formulations of fiber-free basal diet

Ingredients	CO-Free	FO-Free
Corn starch	57.40	57.40
Casein	22.00	22.00
Corn oil	15.00	2.60
Fish oil[1)]	-	12.40
L-methionine	0.30	0.30
AIN 76 mineral mix	4.00	4.00
AIN 76 vitamin mix	1.00	1.00
Choline bitartrate	0.30	0.30
	% nutrients of calculated as calorie	
Total calorie, kcal/100 g diet	452.60	
Protein, %	19.40	
Fat, %	29.80	
Carbohydrate, %	50.70	

1) The FO diet was further supplemented with dl-tocopherol (145.46 mg/100 g of tuna oil).

12h dark. Each rat was intramusculary injected with 1,2-dimethylhydrazine-HCl (DMH, 99%, Aldrich Chemical Co., Milwaukee, WI) twice a week for 6 weeks to give total dose of 180mg/kg body weight. At the same time, an experimental diet was given for 25 weeks and food and water were allowed *ad libitum*. Food intake was recorded at the same time every day and body weight was recorded weekly throughout the study.

Experimental diet was composed of 22% protein, 57.4% carbohydrate, 15% fat, and 6% fiber by weight (Table 1). The major difference in the diet was the types of fat as well as the types of fiber.

We used fish oil extracted from retina of mackerel provided by Dong Won Co, Korea. The corn oil diet contained linoleic acid at 18.0% by calorie and the FO diet contained EPA at 1.35% and DHA at 6.81% by calorie. The fiber-supplemented diets were prepared by uniformly diluting each basal fiber-free diet with the addition of cellulose (powered cellulose PC-200, Pfizer Co) or pectin (powered citrus pectin, Sarnofi Co) at the 6% level. The fish oil diet was supplemented with corn oil to provide a sufficient amount of linoleic acid and supplemented with dl-tocopherol (145.46 mg/100 g tuna oil) to prevent fish oil peroxidation.

2. Sample preparation

At the end of 25 weeks feeding, rats were killed by ethylether and colon resected immediately. The colon segments were rinsed with ice-cold phosphate buffered saline (PBS, pH 7.4) and a count was conducted of tumor-bearing rats and number of tumors. The tumors were cut off horizontally at the height of the noncancerous mucosa and noncancerous mucosa was separated from the muscle. The specimens were stored at $-70℃$ until assay.

3. Biochemical analysis

1) Cell proliferation

To measure in vivo cell proliferation, exactly 1 hour prior to killing, each rat was given an intraperitoneal injection of bromodeoxyuridine (BrdU, Sigma Chemical) at 5 mg/kg body weight. The colon was opened longitudinally and flushed with ice-cold PBS (pH 7.4). A 1cm length of colon was taken from distal segments and placed in 70% ethanol overnight followed by a graded series of ethanol from 80% to 100% and finally xylene prior to embedding in paraffin wax. Paraffin sections of 4 μm thick were cut perpendicular to the mucosal surface and affixed to albumin coated slides. The incorporation of BrdU into DNA was localized using the monoclonal anti-BrdU antibody, and detection of bound antibody was achieved using peroxidase-conjugated antibody to mouse immunoglobin according to the method of Schutte *et al.*[11] A universal peroxidase-staining kit (Signet Laboratories, Dedham, MA) and anti-BrdU mouse antibody (Boehringer Mannheim, Indianapolis, IN) were used to link and label BrdU-incorporated DNA.

2) Polyamines

The Normal mucosa and tumor tissue were homogenized in 0.9% NaCl containing 1,7-diaminoheptane (Internal standard, Sigma Chemical).[12] The supernatant after protein precipitation with 0.5M perchloric acid was dansylated and filtered with a Millipore filtration kit (0.45 μm pore size). Dansylated polyamines were analyzed using high performance liquid chromatograph (Young In, Korea) with Bondapak C18 reverse-phase column (0.46 mm ID × 25 m, 0.45 μm) and a fluorescence detector (excitation 390 nm, emission 400 nm).[12] The analytical conditions were as follows: linear gradient from methanol: water (60 : 40, v/v) to methanol (100%) in 25 min. Flow rate was 0.5 ml/min for 12 min and 1.0 ml/min after 12 min. Dansylated polyamines were identified by comparing the relative retention times of standards.

3) Eicosanoids

One hundred mg of normal colonic mucosa and tumor tissue were homogenized in 0.05M Tris-buffer (pH 8.0, 0.25M sucrose, 1 mM EDTA) containing indomethacin (10 μg/ml, Sigma Chemical). This mixture was incubated in 37℃ shaking water bath for 30 min. and terminated with adding of cold ethanol (final concentration: 15%). The incubated products were acidified to pH 3.0-3.5 with 1M citric acid and centrifuged at 4,000 × g for 15

min at 4℃. The supernatant was passed through C18 solid phase extraction column and extracted with methyl formate. The PGE_2 and TXB_2 were determined using [^{125}I]-PGE_2 and [^{125}I]-TXB_2 radioimmunoassay kit (Du Pont/NEN Research Products, Boston, MA) according to the manufacturer's instruction.

4) Fatty acid composition

The total lipid of normal colonic mucosa and tumor tissue was extracted by the method of Bligh and Dyer[18] and methylated.[19] Fatty acid methyl esters (FAMEs) were extracted with hexane and analyzed by gas chromatograph (Hewlett Packard model 5890 II) equipped with SP 2330-fused silica capillary column (60 m × 0.25 mm, film thickness 0.2 μm) and a flame ionization detector. The analytical conditions were as follows: initial temperature 180C for 4 min followed by a 2℃/min rise to a final temperature of 240℃ for 5 min, injector and detector temperatures 245℃, gas flow rate: N_2 29 ml/min, air 300 ml/min, H_2 30 ml/min, split ratio: 100 : 1.

4. Statistical analysis

All the data were analyzed to determine the effect of dietary fiber, fat and fiber x fat interaction by two-way analysis of variance (ANOVA) using the statistical analysis system and significance for mean was tested using Duncan's multiple range test of general linear model at $p < 0.05$ level.[15]

Table 2. Colon tumor incidence in DMH-treated rats fed different dietary fats and fibers

Groups	(n)	Rats with tumors	Frequency (%)	Total tumors	Average tumors per rat
CO-Free	24	6	25.00	10	0.42±0.18
CO-Cellulose	21	5	23.81	11	0.52±0.24
CO-Pectin	21	3	14.29	4	0.19±0.12
FO-Free	20	2	10.00	2	0.10±0.07
FO-Cellulose	21	2	9.52	2	0.10±0.05
FO-Pectin	21	2	9.52	3	0.14±0.07

(n): Number of rats. Mean ± SE

RESULTS

1. Food intake and weight gain

Food intake and weight gain during experimental period were not significantly different among the groups, suggesting that the observed effects were not the result of differences in the food intake or in the rate of growth.

2. Tumor incidence

Regardless of the type of fiber, the incidence of colon tumors, the number of tumors and average tumors per rat were lower in the FO-fed groups than in the CO-fed groups (Table 2). Tumor incidence was lower by pectin only in the CO-fed groups but not different in the FO-fed groups based on the type of dietary fiber.

3. Fatty acid composition

Overall fatty acid profile of normal colonic mucosal lipid reflected fatty acid pattern of dietary fats, but the relative percentages of AA of the CO-fed groups were higher than those of the FO-fed groups (Table 3). In contrast, the level of DHA of the FO-fed groups was significantly higher than those of the CO-fed groups. Dietary fibers did not have a significant effect on fatty acid profile of colonic mucosa. Specifically, the level of AA in tumor tissue was strikingly higher compared with normal colonic mucosa (Fig. 1).

4. Eicosanoid levels of colonic mucosa and tumor

The levels of PGE_2 and TXB_2 were significantly affected by dietary fats except for pectin supplemented diet for

Table 3. Effect of dietary fat and fiber on fatty acid composition of normal colonic mucosal lipids in DMH-treated rats

Fatty acids	CO-Free	CO-Cellulose	CO-Pectin	FO-Free	FO-Cellulose	FO-Pectin
C18 : 2n6	28.80 ± 4.47^a	31.83 ± 4.91^a	34.72 ± 5.05^a	1.07 ± 0.87^b	0.33 ± 0.13^b	1.02 ± 0.66^b
C18 : 3n3	0.12 ± 0.04^b	0.12 ± 0.03^b	0.13 ± 0.04^b	1.86 ± 1.02^a	0.38 ± 0.09^b	0.79 ± 0.31^{ab}
C18 : 4n6	0.57 ± 0.19^a	0.45 ± 0.21^{ab}	0.36 ± 0.09^{ab}	0.13 ± 0.05^b	0.22 ± 0.06^{ab}	0.11 ± 0.04^b
C20 : 2n6	4.71 ± 2.62	4.28 ± 3.25	5.11 ± 3.55	1.53 ± 0.58	4.24 ± 2.12	4.28 ± 2.81
C20 : 2n6	4.71 ± 2.62	5.11 ± 3.55	4.24 ± 2.12	4.28 ± 3.25	1.53 ± 0.58	4.28 ± 2.81
C20 : 4n6	1.13 ± 0.15^{ab}	1.18 ± 0.17^{ab}	2.29 ± 1.11^a	0.17 ± 0.17^b	0.00 ± 0.00^b	0.46 ± 0.35^b
C20 : 5n3	0.00 ± 0.00^b	0.05 ± 0.05^b	0.08 ± 0.08^b	0.19 ± 0.05^{ab}	0.39 ± 0.14^{ab}	0.77 ± 0.51^a
C22 : 4n6	0.21 ± 0.11	0.33 ± 0.18	0.15 ± 0.08	0.49 ± 0.26	0.08 ± 0.07	0.63 ± 0.47
C22 : 5n3	0.30 ± 0.13	0.09 ± 0.06	0.09 ± 0.07	0.08 ± 0.07	0.11 ± 0.07	0.24 ± 0.15
C22 : 6n3	0.09 ± 0.06^b	0.07 ± 0.05^b	0.07 ± 0.05^b	1.03 ± 0.12^a	1.06 ± 0.17^a	0.67 ± 0.20^{ab}
Σn6	35.41 ± 2.59^a	38.90 ± 1.76^a	41.75 ± 3.04^a	6.13 ± 3.60^b	2.16 ± 0.77^b	6.50 ± 3.79^b
Σn3	0.51 ± 0.20^b	0.34 ± 0.18^b	0.36 ± 0.13^b	3.27 ± 1.12^a	1.95 ± 0.39^{ab}	2.46 ± 1.01^a

Mean ± SE, n = 7. Expressed as relative % of total fatty acids. Means with common superscript in the same raw are not significantly different at $p <$ 0.05 by Duncan's multiple range test.

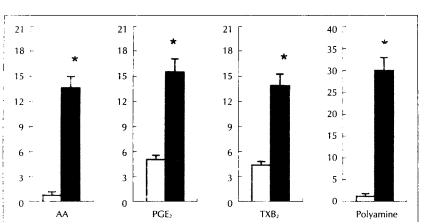

Fig. 1. Arachidonic acid, eicosanoids and polyamines of tumor and surrounding colonic mucosa in DMH-treated rats. AA: arachidonic acid (%), PGE_2: prostaglandin E_2 (ng/mg tissue), TXB_2: thromboxane B_2 (ng/mg tissue), Polyamine (nmole/mg tissue). ★: Significant ag $p < 0.05$.

Table 4. Effect of dietary fats and fibers on colonic mucosal levels of thromboxane B_2 and prostaglandin E_2 in DMH-treated rats

Groups	Thromboxane B_2	Prostaglandin E_2
	ng/mg tissue	
CO-Free	7.46 ± 1.65[a]	20.00 ± 4.33[a]
CO-Cellulose	7.98 ± 1.58[a]	12.39 ± 1.89[b]
CO-Pectin	5.64 ± 0.90[ab]	14.17 ± 3.19[ab]
FO-Free	3.58 ± 0.24[c]	5.93 ± 0.91[c]
FO-Cellulose	3.45 ± 0.63[c]	6.84 ± 1.12[c]
FO-Pectin	5.57 ± 1.20[ab]	7.67 ± 1.08[c]

Mean ± SE. n = 7.
Means with different superscripts in the same column are significant at $p < 0.05$.

Table 5. Effect of dietary fats and fibers on polyamines levels of colon mucosa in DMH-treated rats

Group	Putrescine	Spermidine	Spermine	Total po-
		ng/mg tissue		
CO-Free	0.15 ± 0.06	0.51 ± 0.17	0.45 ± 0.26	1.11 ± 0.47
CO-Cellulose	0.21 ± 0.10	0.55 ± 0.19	0.50 ± 0.26	1.26 ± 0.37
CO-Pectin	0.43 ± 0.27	0.54 ± 0.16	0.37 ± 0.15	1.34 ± 0.43
FO-Free	0.19 ± 0.12	0.34 ± 0.16	0.35 ± 0.20	0.88 ± 0.45
FO-Cellulose	0.16 ± 0.08	0.40 ± 0.21	0.41 ± 0.23	0.96 ± 0.52
FO-Pectin	0.47 ± 0.25	0.54 ± 0.08	0.56 ± 0.07	1.57 ± 0.31

Mean ± SE. n = 7.

TXB_2, but not consistently changed by dietary fiber (Table 4). In the groups of fiber-free and cellulose-fed rats, the normal colonic mucosal levels of PGE_2 and TXB_2 were significantly reduced by FO diet, but only PGE_2 level was significantly reduced by FO diet in pectin-fed rats. The levels of TXB_2 and PGE_2 in tumor tissues were significantly higher than in normal mucosa (Fig. 1).

5. Polyamines

The levels of polyamines in colonic mucosa were not significantly affected by the type of dietary fats and fibers (Table 5), but the level of polyamines in tumor tissue was several-fold higher than those of surrounding normal mucosa (Fig. 1).

Table 6. Effect of dietary fats and fibers on cell kinetic indices in the distal colon of DMH-treated rats

Groups	Crypt length	Total cell per crypt	Labeling index
CO-Free	30.08 ± 3.54	735.11 ± 136.34	21.30 ± 15.66
CO-Cellulose	33.25 ± 6.46	857.38 ± 359.49	19.68 ± 5.47
CO-Pectin	30.19 ± 7.69	780.58 ± 275.93	17.51 ± 4.42
FO-Free	26.86 ± 1.01	627.13 ± 75.01	17.67 ± 2.90
FO-Cellulose	30.91 ± 5.56	771.55 ± 170.83	9.72 ± 4.61
FO-Pectin	33.18 ± 4.16	790.98 ± 203.39	17.92 ± 5.52

Mean ± SE, n = 5. Crypt length = total number of cells in each crypt.
Crypt circumference = number of cells in circumference of each crypt.
Total cells per crypt = crypt length x crypt circumference.
Labeling index = (total number of labeled cells/crypt length) × 100.

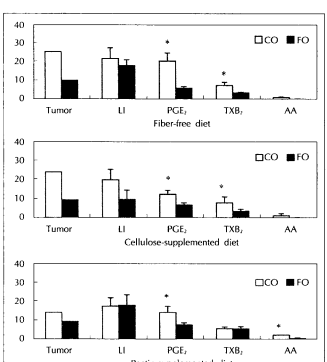

Fig. 2. Comparison of tumor incidence and other biomarkers between CO and FO diet in different fiber-fed rats. CO: corn oil, FO : fish oil, Tumor: tumor incidence (%), LI: labeling index (%), PGE_2: prostaglandin E_2 (ng/mg tissue), TXB_2: thromboxane B_2 (ng/mg tissue), AA: arachidonic acid (%), ★: Significant at p 0.05.

6. Cell proliferation

Dietary fiber and fat did not have a significant effect on cell kinetic indices in the crypt of the distal colon (Table 6).

DISCUSSION

The present study showed that DHA-rich fish oil reduced colon tumor incidence compared with corn oil. As reported previously,[16] n-3 fatty acid of fish oil inhibited tumor development relative to n-6 fatty acid of corn oil. It was reported that n-3 fatty acid reduced AA synthesis by competitive binding for desaturase and elongase with n-6 fatty acid and also reduced the incorporation of AA into membrane phospholipid.[17] Such changes in the balance of n-3 and n-6 PUFA content in membrane phospholipid may affect the physiologic properties of the membrane and its bound enzymes, which regulate the sensitivity of cells to carcinogenic stimuli and tumor growth.[18] In our study, major fatty acid composition of mucosal total lipid was significantly affected by the type of dietary fat but not by fiber. Fish oil-derived n-3 fatty acid (DHA) was easily incorporated into the membrane of the colonic mucosal, which may have been induced by a decrease in the level of AA and competition with AA for binding site in cyclooxygenase, resulting in a reduction of AA-derived prostaglandins. It was confirmed in the present experiment that fish oil significantly reduced the levels of PGE_2 and TXB_2 in normal colonic mucosa with a lower frequency of colon tumor (Fig. 1), which correlated with the decrease of AA (%) in the mucosal membrane. These results indicate that the anti-tumor effect of fish oil resulted from the reduced sensitivity of colonic epithelial cells due to low levels of PGE_2 and TXB_2. An increased synthesis of PGE_2 and TXB_2 might has a potential role in the modulation of colonic cell proliferation and promote colon carcinogenesis (Fig. 2). According to several studies of Lupton and Chapkin,[19] fish oil inhibited tumor incidence through the change of cell response by decreasing cell proliferation, signal transduction by diacylglycerol, protein kinase C activation, and secondary bile acids excretion, etc. Also, in a recent study, they[20] reported that there was no effect of fat or fiber on the number of proliferative cells/crypt column in either the proximal or distal colon. In contrast, fish oil resulted in a greater degree of differentiation compared with corn oil in both colonic sites. In addition, fish oil resulted in a higher number of apoptotic cells/crypt column in both the proximal and distal colon as compared with corn oil. However, cell proliferation was not affected by dietary fat and fiber in our study (Fig. 2). It might be that anti-tumor effect of fish oil was related to the change of cell response caused by AA-derived eicosanoid.

Grande *et al.*[21] said that colonic tumor incidence was significantly lower in indomethacin-treated rats than in untreated control rats and administration of indomethacin also suppressed carcinogenesis through inhibiting ODC induction in large bowel mucosa. This indicated that PGE_2 and TXB_2 played an important part in ODC induction and the development of tumor promoting activity. Marked increases in ODC activity and the rapid accumulation of polyamines are early events associated with cellular proliferation and growth of tissues.[7] Although the level of polyamine in this study was significantly higher in tumor tissue compared with those of surrounding normal mucosa (Fig. 1), it was not different in normal tissue by the type of dietary fats and fibers. This suggests that the difference in polyamine level between tumor tissue and normal colonic mucosa was a gradient increase in a stepwise manner from the normal colonic mucosa to benign lesions such as familial or non-familial polyps to adenocarcinoma. From the above results, it seemed that the protective effect of fish oil against colon carcinogenesis might be due to a decrease in tumor promoting process and tumor growth through an inhibition of cellular signal transduction by 2-series eicosanoids and polyamines and an increase in apoptosis and differentiation rather than a decrease in proliferation.

Although it was reported that insoluble fiber such as cellulose could inhibit colon carcinogenesis and that soluble fiber such as pectin could promote it,[22] the effect of fiber on colon carcinogenesis in this study showed conflicting results depending on the type of dietary fat in the diet. Tumor incidence was reduced by pectin only when corn oil was used as a fat source, while its reduction was not observed when fish oil was used as a fat source. Nevertheless, it was reported that dietary fiber could have a potential effect on PG synthesis by influencing on bile acid excretion because bile acids have been shown to enhance PGE_2 release from the colon.[23] Secondary bile acids such as deoxycholic acid and lithocholic acid stimulated colonic epithelial cell proliferation through their enhancing effects on colonic production of PGs by activation of phospholipases and release of free AA.[24] However, in this study, dietary fiber did not have a significant effect on cell proliferation, the levels of polyamines and eicosanoids, and fatty acid profile even though tumor incidence was decreased by pectin in corn oil-fed rats,

which might not be a potential effect of dietary fiber on colon carcinogenesis.

CONCLUSION

Fish oil markedly decreased tumor incidence and significantly reduced the relative percentage of AA but increased that of DHA in normal colonic mucosa, which resulted in the reduction of PGE_2 and TXB_2 synthesis in normal colonic mucosa. The levels of polyamines, eicosanoid and AA levels in tumor tissues were significantly increased compared to those in normal colonic mucosa. However, we did not observe significant effect of dietary fiber on tumor incidence, cell proliferation, polyamines, AA and eicosanoids levels. We also found a lack of consistency regarding the effects of fibers on colon tumorigenesis.

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