Effect of Dietary Eicosapentaenoic Acid on Azoxymethane-induced Colon Carcinogenesis in Rats

Toshiyuki Minoura, Toshiyuki Takata, Michitomo Sakaguchi, Hideho Takada, Manabu Yamamura, Koshiro Hioki, and Masakatsu Yamamoto

Department of Surgery, Kansai Medical University, 1 Fumizono, Moriguchi, Osaka, 570, Japan

ABSTRACT

The effects of eicosapentaenoic acid (EPA, n-3 polyunsaturated fatty acid) and linoleic acid (n-6 polyunsaturated fatty acid) on azoxymethane-induced colon carcinogenesis in rats were studied. Male Donryu rats were given two types of semipurified diet containing 4.7% EPA plus 0.3% linoleic acid and 5% linoleic acid. The rats were given s.c. injection of azoxymethane (7.4 mg/kg body weight once a week for 11 weeks) and sacrificed 15 weeks after the last injection of azoxymethane. The tumor incidence and tumor yields (tumors per rat) of the colon were significantly lower in rats on the EPA diet compared to those on the linoleic acid diet; i.e., 33%, 0.41 ± 0.61 and 69%, 1.66 ± 1.69, respectively. In the analysis of phospholipid fatty acid composition, the colon tumor showed higher levels of arachidonic acid and lower levels of linoleic acid than those in the normal colon mucosa in both diet groups. Despite the increase of arachidonic acid in colon tumor, the EPA diet suppressed the excessive production of prostaglandin E\(_2\), which may be accompanied with neoplastic formation, whereas linoleic acid diet caused a marked increase in the tumor content of prostaglandin E\(_2\) compared to normal colon mucosa. These results suggest that EPA exerts its inhibitory effect on colon carcinogenesis by modulating lipid metabolism and inhibiting prostaglandin E\(_2\) synthesis in tumor cells.

INTRODUCTION

Epidemiological data have indicated that dietary factors, especially the amount of fat and animal fat, are closely related to the risk of colon and mammary cancer rather than the degree of unsaturation (1, 2). However, Alaskan and Greenland Eskimos characteristically have had low incidences of cancer, considering their total fat intake which has always been comparable with high-risk Danish levels, and the upward shift of their cancer risk has been associated well with increased urbanization and westernization (3–5). Alaskan and Greenland Eskimos have consumed large amounts of fat derived from marine oil which contains n-3 polyunsaturated fatty acids, mainly EPA\(^1\) (C20:5) and DHA (C22:6) (6). This is attributed to the low incidence of ischemic heart disease (7) which well correlates with colon cancer incidence (8).

On the other hand, in experiments on rodents, n-6 polyunsaturated fatty acids, especially linoleic acid, have been shown to promote colon (9, 10), mammary (11, 12), and pancreas (13) carcinogenesis. Linoleic acid is desaturated and provides arachidonic acid in cellular phospholipids which is the precursor of PG. Our previous study indicated that an excess of arachidonic acid, or its metabolites, may act as a cocarcinogen of colon cancer (10). In fact, excessive production of PG was observed in various malignant cells (14), and various inhibitors of PG synthesis, such as indomethacin, aspirin, and eicosatraynoic acid suppressed tumor growth and carcinogenesis in numerous experimental models (15–18). EPA and DHA also act as competitive inhibitors of cyclooxygenase, the first enzyme for PG synthesis (19, 20). Karmali et al. (21) reported that administration of MaxEPA had an inhibitory effect on the growth of R3230AC transplantable mammary tumors by inhibiting 2-series PG synthesis in tumor. O’Connor et al. (22) found that a 20% menhaden oil diet significantly reduced the size and number of 1-azaserine-induced preneoplastic lesions of the pancreas compared to a 20% corn oil diet. Recently, Reddy et al. (23) showed that intake of high Menhaden oil diet reduced AOM-induced colon carcinogenesis when compared to high corn oil diet.

The question has been raised as to which component of fish oil exerts this antitumor effect. EPA is the precursor of 3-series PG, the biological role of which is yet unknown, but DHA is not a precursor of 3-series PG. Moreover, commercial fish oil contains other fatty acids besides EPA and DHA at various levels. In this study, the effect of purified EPA on AOM-induced colon carcinogenesis were compared with those of linoleic acid in rats. To shed light on the underlying mechanism, the alteration and difference of fatty acid composition and the content of PGE\(_2\) in colon tumor and normal colon mucosa were also studied.

MATERIALS AND METHODS

Fatty Acids. EPA ethyl ester refined from fish oil was kindly supplied by Nishin Flour Milling Co., Ltd., Tokyo, Japan, and linoleic acid ethyl ester was kindly supplied by Ono Pharmaceutical Co., Osaka, Japan. The purity of fatty acid determined in our laboratory was as follows: EPA (91.1% 5,8,11,14,17-eicosapentaenoic acid C20:5, 3.9% arachidonic acid C20:4, 3.5% 8,11,14,17-eicosatetraenoic acid C20:4), linoleic acid (91.5% linoleic acid C18:2, 7.7% oleic acid C18:1).

Animals and Diets. Weanling male Donryu rats each weighing 100–115 g (5 weeks old) were purchased from Laboric Service Co., Shiga, Japan. The basal fat-free semipurified diets were prepared at Oriental Yeast Co., Tokyo, Japan, supported by Eisai Co., Tokyo, Japan. Table 1 shows the composition of the experimental diets. Two types of 5% fat diets were prepared in our laboratory every other day and stored in the dark at 4°C. 0.3% (v/v) linoleic acid was added to 4.7% (v/v) EPA diet to keep good growth without deficiency of essential fatty acid (10).

Experimental Method. Following a 1-week period of adaptation, 100 rats were randomly divided into four groups. Forty rats each were fed the EPA diet or linoleic acid diet and given the carcinogen, and 10 rats in each dietary group were served as control. Azoxymethane (Ash Stevens Inc., Detroit, MI) was dissolved in 0.9% NaCl solution and injected s.c. into the inguinal region once a week for 11 weeks at a concentration of 7.4 mg/kg of body weight. An equal volume of normal saline was given to the controls. Fifteen weeks after the last injection of AOM, 30 rats treated with AOM in both dietary groups were sacrificed 15 weeks after the last injection of azoxymethane. DHA, 4,7,10,13,16,19-docosahexaenoic acid; PG, prostaglandin(s); PGE\(_1\), prostaglandin E\(_1\); PGE\(_2\), prostaglandin E\(_2\).

Received 1/19/88; revised 4/28/88; accepted 6/6/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by a Grant-in-Aid (61570626) for Scientific Research of the Ministry of Education, Science and Culture of Japan.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: EPA, 5,8,11,14,17-eicosapentaenoic acid; AOM, azoxymethane; DHA, 4,7,10,13,16,19-docosahexaenoic acid; PG, prostaglandin(s); PGE\(_1\), prostaglandin E\(_1\); PGE\(_2\), prostaglandin E\(_2\).

4790
The antigen-antibody complex was precipitated with 16% polyethylene glycol (PEG6000) solution. Radioactivity in the precipitate was counted in a gamma counter. The cross-reactivity of PGE2 antibody to PGE1 was less than 4%.

Statistical Analysis. Tumor incidence and histological differences were analyzed with the $\chi^2$ method, and other data were analyzed with Student's t test.

RESULTS

General Observations. All rats were weighed weekly, and 20-h food consumption was measured once a month. None of the rats died during the experiment except for one rat treated with AOM in the linoleic acid diet group, which died at 3 weeks of the experiment. Autopsy revealed no specific change other than pneumonia. There were no signs of essential fatty acid deficiency in the EPA diet group. On the other hand, the rats on the EPA diet were 10–15% smaller than those given the linoleic acid diet from 1 week after the start of experimental diet (Fig. 1), although the food consumption was not significantly different between the two diet groups. The lungs, liver, spleen, and kidneys were examined histologically at autopsy, but there were no specific changes associated with the diet or carcinogen treatment.

Tumor Incidence. There were no colon tumors in the vehicle-treated rats. Table 2 summarizes the AOM-induced colon tumor incidence and yield in rats given the EPA and linoleic acid diets. The rats fed the EPA diet had a significantly lower tumor incidence and number of tumors per rat than those fed the linoleic acid diet. The ratio of colon tumors in the proximal half of the colon to total colon tumors was much higher ($P < 0.01$) in the EPA diet group (10/12) than the linoleic acid diet group (18/48). The diameter and depth of invasion of colon tumors were not significantly different between the two groups. Histological examination showed that the rats on the EPA diet had fewer well-differentiated adenocarcinomas and more mucinous adenocarcinomas than those on the linoleic acid diet as shown in Table 3. However, invasion of the colon tumor to the regional lymph nodes, liver and diaphragm was observed in two rats on the linoleic acid diet. Tumors of the small intestine were also observed in two rats on the linoleic acid diet, but not in those on the EPA diet.

Phospholipid Contents. The contents of phospholipids and protein of colon tumors were higher than those of normal colon mucosa in both diet groups (Table 4). The content of phospholipids in tissue was not affected by the diets.

Fatty Acid Composition. Table 5 shows phospholipid fatty acid composition of colon tumors and normal colon mucosa in rats on the EPA and linoleic acid diets. The overall composition of fatty acid reflected essentially the fatty acid ingested, but the fatty acid composition in normal colon mucosa was similar in the two groups except for the increase of the proportion of C18:0 in EPA diet group. However, in colon tumors, the proportion of C16:0 and C20:4 was higher, and the proportion of C18:0 and C18:2 was lower than that of each paired normal colon mucosa. Furthermore, the contents of C18:2 and C20:4 in colon tumors were significantly lower in the EPA diet group than in the linoleic acid diet group.

Table 6 shows the neutral lipid fatty acid composition of colon tumors and normal colon mucosa in rats on the EPA and linoleic acid diet. Neutral lipid fatty acid composition was affected more directly by lipid ingredient ingested and reflected lipid metabolism when compared to phospholipid fatty acid composition. Grossly, normal colon mucosa in the EPA diet group had a higher proportion of chain-shortened fatty acids.
Table 2 Colon tumors in azoxymethane treated rats fed EPA and linoleic acid diets

<table>
<thead>
<tr>
<th>EPA diet</th>
<th>Linoleic acid diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>No. of rats</td>
</tr>
<tr>
<td>30</td>
<td>100 (93)</td>
</tr>
<tr>
<td>29</td>
<td>20 (69)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from linoleic acid diet group (P < 0.01) by χ² analysis.
<sup>b</sup> Significantly different from linoleic acid diet group (P < 0.001) by Student's t test.
<sup>c</sup> Mean ± SD of three or more samples.
<sup>d</sup> Significantly different from linoleic acid diet group (P < 0.05) by χ² analysis.

Table 3 Histology of colon tumors in azoxymethane treated rats fed EPA and linoleic acid diets

<table>
<thead>
<tr>
<th>No. of Colon tumors</th>
<th>EPA diet</th>
<th>Linoleic acid diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-differentiated adeno-carcinoma</td>
<td>3&lt;sup&gt;f&lt;/sup&gt; (25.0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Moderately differentiated adeno-carcinoma</td>
<td>10 (33.3)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Poorly differentiated adeno-carcinoma</td>
<td>3&lt;sup&gt;f&lt;/sup&gt; (41.7)</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from linoleic acid diet group (P < 0.05) by χ² analysis.
<sup>b</sup> Significantly different from paired controls (P < 0.01).
<sup>c</sup> Mean ± SD of three or more samples.
<sup>d</sup> Significantly different from linoleic acid diet group (P < 0.01).

Table 4 Phospholipid and protein contents of colon tumors and normal colon mucosa in rats fed EPA and linoleic acid diets

<table>
<thead>
<tr>
<th></th>
<th>Phospholipid (µmol/g wet tissue)</th>
<th>Protein (mg/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon tumors</td>
<td>11.8 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.9 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal colon mucosa</td>
<td>7.3 ± 1.7</td>
<td>36.5 ± 5.0</td>
</tr>
<tr>
<td>Linoleic acid diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon tumors</td>
<td>11.8 ± 1.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.1 ± 2.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal colon mucosa</td>
<td>7.2 ± 0.9</td>
<td>37.9 ± 2.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD of three or more samples.
<sup>b</sup> Significantly different from paired controls (P < 0.01).
<sup>c</sup> Significantly different from paired controls (P < 0.05).

than the linoleic acid diet group. However, the colon tumors had a higher proportion of C18:1 and lower proportion of C16:1 than each paired normal colon mucosa. On the other hand, the contents of C18:2 and C20:4 of colon tumors in the linoleic acid diet group were significantly higher than those of paired normal colon mucosa and colon tumors in the EPA diet group.

PGE<sub>2</sub> Content. The content of PGE<sub>2</sub> in the colon tumor in the linoleic acid diet group was markedly higher than that of paired control normal colon mucosa and that in the colon tumors in the EPA diet group. In spite of the higher content of arachidonic acid, the colon tumor content of PGE<sub>2</sub> in the EPA diet group was comparable to that of normal colon mucosa in both diet groups as shown in Table 7.
DISCUSSION

Animal model studies have shown that not only the amount of dietary fat but also the fatty acid composition (type) of fat are important determinants in colon carcinogenesis (9, 33). In our previous study, at low levels of dietary fat (5%), the unsaturated fatty acid (linoleic acid) diet had greater effect than the saturated fatty acid (stearic acid) diet in promoting AOM-induced colon cancer (10). The results of the present study show that EPA has an inhibitory effect on colon carcinogenesis compared to linoleic acid in the same experimental model, confirming the findings of Reddy et al. (23).

In biological membranes, the phospholipid composition, especially unsaturated fatty acid, determines the functional specificity, including prostanoids synthesis, and the membrane structure which influences fluidity and lipid-protein interactions (34). Thus, EPA may have more nutritional importance than saturated fatty acid in colon carcinogenesis because of its influence on the metabolism of linoleic acid and its derivatives, and of cholesterol including bile acids. Furthermore, EPA is not synthesized de novo.

In the present study, the linoleic acid diet markedly increased the proportion of linoleic acid in both neutral lipid and phospholipid fractions of colon tumors and normal colon mucosa, whereas the EPA diet only increased the chain-shortened fatty acids. Wong et al. (35) reported that lipogenesis was decreased and ketogenesis (and hence fatty acid oxidation possibly in peroxisomes) was increased to a significantly greater degree in the liver of fish oil-fed rats than in the liver of rats fed safflower oil, which mainly contains linoleic acid. And oxidation in peroxisome has been shown to yield chain-shortened fatty acids (36). Such evidence could account for the change of fatty acid composition by the EPA diet and also the loss of body weight of rats on the EPA diet in our study.

The marked change could be the decrease of linoleic acid and the increase of arachidonic acid in the proportion of phospholipid fatty acid composition of colon tumors in both diet groups. Such changes have been observed in chemically induced mammary tumor (37). The turnover of linoleic acid with respect to its conversion to arachidonic acid may have been increased during neoplastic formation. However, EPA may in part displace n-6 fatty acids in colon tumor because the ratio of n-3 fatty acids to n-6 fatty acids in colon tumor was greater compared to that in normal colon mucosa. Moreover, EPA is reported to inhibit the conversion of linoleic acid to arachidonic acid (38). Arachidonic acid is released from cellular phospholipids by the action of phospholipase A, and is a precursor of 2-series PG. Bennett et al. (39) observed excessive amounts of PG in human colon cancer and Narisawa et al. (17) showed that indomethacin, a cyclooxygenase inhibitor, has an inhibitory effect on methyl linoleate-induced rat colon tumors. The present findings suggest that the EPA diet suppresses the excessive production of PGE in colon tumor, which may be accompanied with neoplastic formation, by inhibiting cyclooxygenase competitively (19).

PGE and cyclic 3',5'-monophosphate, which is increased by PGE, has been assumed to have a possible integral role in cellular proliferation and differentiation as well as tumor dissemination (40). Prasad (41) found that exogenous treatment of PGE induced morphological differentiation in mouse neuroblastoma cells and Bennett et al. (42) showed that well-differentiated adenocarcinoma yielded more amounts of prostaglandin-like material than undifferentiated carcinoma in human lung cancer. These observations are supported by the present findings that the colon tumors in the EPA diet group contained less PGE than those in the linoleic acid diet group, and consisted of a higher proportion of mucinous adenocarcinoma and a lower proportion of well-differentiated adenocarcinoma. PG production is elevated when active tumor invasion proceeded in human breast cancer (43). This could explain the two cases of spreading colon tumor in linoleic acid diet group.

The involvement of PG in cancer may be the modulation of cellular and humoral immune responses. PGE has been shown to inhibit lymphocyte proliferation, lymphokine production such as interleukin 2 (44), antibody production by B-lymphocytes (45), natural killer activity and macrophage-mediated cytotoxicity to cancer cell (46, 47) in vitro. These could be restored by the administration of a PG synthesis inhibitor such as indomethacin. Recently, Alexander et al. (48) reported that fish oil administration resulted in better cell-mediated immune response and better opsonic indices when compared to safflower oil as well as linoleic acid alone after burn injury in guinea pigs. Considering these results, EPA may exert an inhibitory effect on colon carcinogenesis in rats by enhancing general or local host immune responses.

Another mechanism may involve bile acid (colon tumor promoter) metabolism. Linoleic acid increases the excretion of fecal secondary bile acids, resulting in promoting colon carcinogenesis (9, 49). Fish oil has been suggested to reduce the plasma cholesterol level in rats by increasing the transfer of cholesterol into bile without an increase of bile acid secretion (50), which may affect bile acid metabolism. Recently, Nasisawa et al. (51) showed that deoxycholate treatment increased the amount of ornithine decarboxylase (a marker of tumor promotion) activity by stimulating PGE synthesis in rat colon mucosa. However, PGE itself did not have the effect on the ODC activity. This suggests that the colonic concentration of secondary bile acid is reduced in tumor-bearing rats on the EPA diet.

On the other hand, the body weight loss of rats on the EPA diet might in part have contributed to the reduction of colon tumor incidence (52), although the food consumption or caloric intake was comparable to that of the linoleic acid diet group. Reddy et al. (23) have also observed similar weight loss in rats on high Menhaden oil diet. The effects of lowering serum triglyceride and cholesterol by fish oil have been recognized. It is possible that the nutritional effect associated with the change of neutral lipid metabolism caused by the EPA diet will affect the colon carcinogenesis. Thus, further studies are needed to understand the complex involvement of EPA on colon carcinogenesis.

ACKNOWLEDGMENTS

The authors thank Dr. M. Ogura, Department of Pathology of this university, for his help in histological evaluation and Nisshin Flour Milling Co. Ltd., Tokyo, Japan, for kindly providing EPA.

REFERENCES

18. Carter, C. A., Millholland, R. J., She, W., and Ip, M. M. Effect of prosta
33. Sakaguchi, M., Minoura, T., Hiramatsu, Y., Takada, H., Yamamura, M., Hicki, K., and Yamamoto, M. Effects of dietary saturated and unsaturated fatty acids on fecal bile acids and colon carcinogenesis induced by azoxy
Effect of Dietary Eicosapentaenoic Acid on Azoxymethane-induced Colon Carcinogenesis in Rats

Toshiyuki Minoura, Toshiyuki Takata, Michitomo Sakaguchi, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/17/4790

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.