Effect of Dietary Fish Oil on Azoxymethane-induced Colon Carcinogenesis in Male F344 Rats1

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ABSTRACT

The effect of dietary intake of different levels of Menhaden fish oil on azoxymethane-induced carcinogenesis was examined in male F344 rats fed the semipurified diets. Starting at 5 weeks of age, groups of animals were fed the 5% corn oil (low corn oil) diet. At 7 weeks of age, all animals except the vehicle-treated controls were given s.c. injections of azoxymethane (15 mg/kg body weight/week for 2 weeks). After 4 days, groups of animals were fed the diets containing 4% Menhaden oil + 1% corn oil (low fish oil), 22.5% Menhaden oil + 1% corn oil (high fish oil), 5% corn oil, and 23.5% corn oil (high corn oil). Thirty-four weeks after azoxymethane injections, all animals were necropsied. High fish oil diet had no tumor promoting effect in the large intestine when compared to the high corn oil diet. There was no difference in large intestinal tumor incidence among the other dietary groups. The results of this study indicate that fish oils rich in highly polyunsaturated n-3 fatty acids do not enhance large bowel carcinogenesis and that the fatty acid composition of the dietary fat is one of the determining factors in large bowel carcinogenesis.

INTRODUCTION

Epidemiological studies have shown that diets particularly high in total fat and animal fat or low in certain fibers are generally associated with an increased risk for colon cancer development (1–6), although a recent prospective study showed no increased effect of dietary saturated fat or total fat in colon cancer (7). Discrepancies in epidemiological studies might have stemmed from methodological problems of dietary assessment, because several of these studies not only rarely distinguished between the types of saturated and polyunsaturated fats consumed but did not take into consideration other confounding factors such as dietary fiber. Several animal model studies demonstrated that high fat diets containing corn oil, safflower oil, lard, or beef tallow enhanced the chemically induced colon tumors in rats, whereas the diets containing high levels of coconut oil, olive oil, or trans-fat had no colon tumor-promoting effect (8–14). However, another study suggests no enhancing effect of dietary beef tallow or corn oil on colon tumors (15). These studies suggest that the fatty acid composition of the fat is an important determining factor in colon tumorigenesis.

Interest in the marine oils emerged from the observation that cancer incidence rates are generally low in Alaskan and Greenland Eskimos compared to American whites and other western populations, despite the fact that these defined populations eat high-fat diets (16–19). Although fish oils are rarely found in western diets, Eskimo diets contain large amounts of oils derived from fish and seals (20–22). The high amount of highly polyunsaturated (n-3 series) fatty acids such as eicosapentaenoic acid (C20:5, n-3) and docosahexaenoic acid (C22:6, n-3) present in fish oils make them unique dietary fats (23, 24). Recent studies in animal models demonstrated that high levels of dietary Menhen fish oil had no promoting effect on 7,12-dimethylbenz[a]anthracene-induced (25) and methyl-trinitrosourea-induced (26) mammary carcinogenesis when compared to high dietary corn oil in rats. Dietary intake of 20% Menhen oil when compared to 20% corn oil produced a significant inhibition in both size and number of preneoplastic lesions in rat pancreas (27). Karmali et al. (28) reported that daily treatment of 0.2 ml Max EPA, a commercially available fish oil, inhibited growth of transplantable mammary tumors in rats. The present study was designed to investigate the modifying effect of dietary fish oil on AOM1-induced large intestinal carcinogenesis in rats.

MATERIALS AND METHODS

Animals, Diets, and Carcinogen. A total of 124 weanling male F344 rats were purchased from Charles River River Laboratories (Wilmington, MA). All semipurified dietary ingredients were from Dyets, Inc. (Bethlehem, PA), and AOM (CAS:25843-45-2) was from Ash-Stevens, Inc. (Detroit, MI). Menhaden fish oil was donated by Zapata Haynie Corporation (Reedville, VA).

Male F344 rats received at weaning were quarantined for 10 days and then randomly assigned into 4 dietary groups of 36 animals each. Each dietary group was divided into AOM-treated (24 animals) and vehicle-treated (12 animals) subgroups and housed 3 to a plastic cage with filter tops in the animal holding room under controlled environmental conditions of a 12-h light-dark cycle, 50% humidity, and 21°C. All animals were fed ad libitum and had free access to water. The food cups were replenished every day.

The composition of experimental semipurified diets is shown in Table 1 and is based on revised AIN-76 diet (29, 30). The composition of high- and low-fat diets was adjusted so that the animals in all dietary groups would consume the same amount of calories, protein, vitamins, minerals, and fiber (14, 31). All diets were prepared in our laboratory 3 times weekly and stored in a cold room at 4°C. Corn oil (1%) was added to low and high Menhaden fish oil diets to provide linoleic acid and to alleviate essential fatty acid deficiency. Freshly prepared diets and those stored for 2 days in a cold room were analyzed for peroxidizability using thiobarbituric acid method (32). There were no detectable levels of peroxides in the diets.

The fatty acid composition of Menhaden fish oil was analyzed and provided by Zapata Haynie Corporation. It contained about 15% palmitic acid (C16:0), 12% palmitoleic acid (C16:1, n-6), 10% oleic acid (C18:1, n-6), 16% eicosapentaenoic acid (C20:5, n-3), 11% docosahexaenoic acid (C22:6, n-3), and 1.8% linoleic acid (C18:2, n-6). Corn oil contains about 10% palmitic acid, 31% oleic acid, and 56% linoleic acid (33). Menhaden fish oil and corn oil contained about 0.2 ml Max EPA, a commercially available fish oil, and 0.2 ml Max EPA, a commercially available fish oil, respectively.

Experimental Procedure. Starting at 5 weeks of age, all animals were fed the experimental diet containing 5% corn oil and continued on this diet until 4 days after carcinogen or vehicle treatment. At 7 weeks of age, all animals intended for carcinogen treatment in each subgroup were given s.c. injections of AOM (15 mg/kg body wt/wk) once weekly for 2 weeks, whereas the animals intended for vehicle treatment were given s.c. injections of vehicle.
weeks on experimental diets indicated that the animals fed the
haden oil diet were comparable throughout the experimental
body weights of animals fed the corn oil diets and low Men
observed starting at 8 weeks on this diet. On the other hand,
body weight of animals fed the high Menhaden oil diet was
less than those fed the other diets (Table 2). The decrease in
according to histológica!criteria (34).Statistical Analysis. The tumor data were analyzed by the 
method, were fixed in 10% buffered formalin and embedded in paraffin. Paraffin-
examined grossly under the dissection microscope for tumors. Tissues
endoscopie examination of the colon reveals the presence and size of
tumors in the lower pari of the colon without sacrificing the animals.
period of 1 week at the end of 12 and 22 weeks on experimental diets.
termination of the experiment. Food consumption was measured for a
weeks post-AOM treatment. Body weights were measured weekly until
at autopsy, all organs, including intestines, were
experiment was terminated 34 weeks after the last AOM injection.

diets containing 4% Menhaden fish oil + 1% corn oil (designated as
low Menhaden oil diet), 22.5% Menhaden fish oil + 1% corn oil (high Menhaden oil diet), or 23.5% corn oil (high corn oil diet). An additional
group consuming the 5% corn oil diet (low corn oil diet) was continued
on the same diet. All animals were fed the experimental diets until
termination of the experiment. The experiment was terminated 34
weeks post-AOM treatment. Body weights were measured weekly until
the animals attained 16 weeks of age and then every 4 weeks until the
termination of the experiment. Food consumption was measured for a
period of 1 week at the end of 12 and 22 weeks on experimental diets.

Four animals treated with AOM from each dietary group were
endoscoped 20 and 30 weeks after the last AOM injection, since
endoscopic examination of the colon reveals the presence and size of
tumors in the lower part of the colon without sacrificing the animals.
Not only were the tumors that were present in animals endoscopically examined at 20 weeks after the last AOM injection also observed at 30
weeks, but also the size of these tumors was increased at 30 weeks. The
experiment was terminated 34 weeks after the last AOM injection.
Both AOM- and vehicle-treated animals were sacrificed by CO2 eutan-
asia as scheduled. At autopsy, all organs, including intestines, were
examined grossly under the dissection microscope for tumors. Tissues
were fixed in 10% buffered formalin and embedded in paraffin. Paraffin-
embedded tissue sections were then stained with hematoxylin and eosin and examined histologically for tumor types. Each tumor was classified
according to histological criteria (34).

Statistical Analysis. The tumor data were analyzed by the χ² method,
Fisher's exact test, and Student's t-test.

RESULTS

Body Weights and Food Consumption. Animals fed the high
Menhaden oil diet and treated with AOM or vehicle weighed
less than those fed the other diets (Table 2). The decrease in
body weight of animals fed the high Menhaden oil diet was
observed starting at 8 weeks on this diet. On the other hand,
body weights of animals fed the corn oil diets and low Men-
haden oil diet were comparable throughout the experimental
period. Food consumption measured at the end of 12 and 22
weeks on experimental diets indicated that the animals fed the
low corn oil or low Menhaden oil diets consumed about 12–
13% more food than those fed the high corn oil or high
Menhaden oil diets. There was no difference in food intake
between low corn oil and low Menhaden oil groups or among
high corn oil and high Menhaden oil groups. Except for the
number of calories from fat in the diet, the intakes of protein,
vitamins, minerals, non-nutritive fiber, and total calories were
similar in all dietary groups.

Tumor Incidences. Table 3 summarizes the AOM-induced large
intestinal tumor incidence and multiplicity in animals fed
various diets. There was no evidence of tumor incidence in
vehicle-treated animals. Large intestinal tumor incidence (num-er of animals with tumors) and large intestinal tumor multi-
plicity (number of adenomas and adenocarcinomas/animal)
were significantly lower in animals fed low and high Menhaden
oil diets and the low corn oil diet than in animals fed the high
corn oil diet. The incidence of large intestinal tumors did not
differ significantly among the groups fed low and high Men-
haden oil diets and the low corn oil diet. There was no statisti-
cally significant difference in the multiplicity of adenomas
and adenocarcinomas between the groups fed the low and high
Menhaden oil diets.

Table 4 shows the AOM-induced tumor incidence in small
intestine and ear duct. Tumors of the small intestine were
adenomas and adenocarcinomas, whereas tumors of the ear
duct were squamous cell carcinomas. Small intestinal tumors
were all localized in the duodenum. Low and high Menhaden
oil diets or low and high corn oil diets had no significant effect
on small intestinal and ear duct tumor incidences.

DISCUSSION

The results of the present study not only confirm our previous
study in female rats (14) that a diet containing high corn oil
significantly increased the AOM-induced large intestinal tumor
incidence and multiplicity compared to a low corn oil diet but
extends our observation that a high Menhaden oil diet had no
tumor enhancing effect in the large intestine compared to a
high corn oil diet. We are not aware of any previous study of a
potential large intestinal tumor inhibitory effect by a fish oil
diet. Recent studies demonstrated that diets containing 20% Menhaden oil induced fewer 7,12-dimethylbenz(α)anthracene-
or methylnitosourea-induced mammary tumors, as well as
produced a significant reduction of the development of both the
size and number of L-azaserine-induced preneoplastic lesions
in the pancreas when compared to a diet containing 20% corn
oil (25, 26, 28).

The lack of large bowel tumor promoting effect of high
dietary Menhaden oil observed in this study and that of high
dietary olive oil, coconut oil, and trans-fat observed in our
previous study (11, 14), in contrast to that of high dietary corn
oil, safflower oil, beef fat, and lard (3, 8) suggests that the fatty
acid composition of a dietary fat is one of the determining
factors in large bowel carcinogenesis. It has been demonstrated
that the excretory pattern of fecal secondary bile acids, namely
deoxycholic acid and lithocholic acid, which have been shown
to act as large bowel tumor promoters, positively correlated
with large bowel tumor incidence in animal models fed various
types and amounts of dietary fat (35). Although the present
study was not designed to address this aspect of the influence
of dietary fish oil on colonic secondary bile acids, it is likely
that high dietary fish oil may have an inhibitory effect on the
colic concentration of secondary bile acids. In addition, we
should not rule out the possibility that the effect of different
types of fat on large bowel carcinogenesis might be mediated
by the active products of essential fatty acids such as prosta-
glandins, since recent studies demonstrated an inhibitory effect of certain prostaglandin synthesis inhibitors (indomethacin) on chemically induced large bowel carcinogenesis in rats (36, 37). In the present study, low and high Menhaden oil diets and low and high corn oil diets contained, respectively, about 0.6, 1.0, 2.8, and 13.2% linoleic acid, a precursor for prostaglandin synthesis. Eicosapentaenoic acid present in Menhaden oil has been shown to be a competitive inhibitor of cyclooxygenase, and high corn oil diets contained, respectively, about 0.6, 1.0, 2.8, and 13.2% linoleic acid, a precursor for prostaglandin synthesis as proposed for mammary carcinogenesis (39). It is possible that the lack of a large bowel tumor promoting effect by Menhaden oil might be due to its inhibitory effect on prostaglandin synthesis as proposed for mammary carcinogenesis (23).

The question also arises as to whether the lack of a large bowel tumor promoting effect of high Menhaden oil diet might be related to the weight loss. Although the food intake was not measured throughout the study, calorie intake measured during 12 and 22 weeks on the experimental diets was similar in all dietary groups. Although a difference in body weights as much as 30% in animals fed various experimental diets did not correlate with large bowel tumor incidence in our previous study (40), it is possible that diminished weight gain in animals fed the high Menhaden oil diet may have contributed to reduced tumor incidence. Additional studies are needed for a better understanding of the overall effect of marine oils in large bowel carcinogenesis.

In conclusion, the present study demonstrates that high dietary Menhaden oil (a) induces fewer large bowel tumors than did the diet containing high corn oil and (2) does not promote large bowel carcinogenesis to any greater extent than does a diet containing either low Menhaden oil or low corn oil.

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