Effects of Dietary Flaxseed on Intestinal Tumorigenesis in Apc\textsuperscript{Min} Mouse

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Dietary flaxseed has been shown to prevent azoxymethane (AOM)-induced colorectal cancers in male Fisher rats. The present study was designed to investigate the chemopreventive effects of dietary flaxseed on the development of intestinal tumors in Apc\textsuperscript{Min} mice. Apc\textsuperscript{Min} mice were divided into five different groups, fed with control (AIN-93M meal), corn meal, flaxseed meal, corn oil, and flaxseed oil supplemented diets. Results showed that dietary flaxseed significantly decreased ($P < 0.05$) tumor multiplicity and size in the small intestine and colon as compared to control, corn-treated groups. Intestine, colon, and serum samples of corn-treated groups showed higher levels of $\omega$-6 fatty acids, whereas the flaxseed treated groups exhibited higher levels of $\omega$-3 fatty acids. Lignans were detected in the serum, intestine, and colon samples for flaxseed meal group. COX-1 and COX-2 expression in the colon samples from the flaxseed meal group were significantly lower ($P < 0.05$) as compared to the corn meal group. Dietary flaxseed may be chemopreventive for intestinal tumor development in Apc\textsuperscript{Min} mice possibly by increasing $\omega$-3 fatty acid levels, lignans, and decreasing COX-1 and COX-2 levels.

INTRODUCTION

In 2008, 108,070 new colon and 40,740 new rectal cases estimated to be diagnosed in the United States and 49,960 deaths were expected from colorectal cancers (CRC), making CRC the third leading cause of cancer deaths in the US (1). Colorectal carcinogenesis has been demonstrated to involve the accumulation of genetic alterations in genes coding for proteins, which are known to function as key players in signaling pathways involved in regulation of apoptosis, cell proliferation, differentiation, angiogenesis, and metastasis (2). CRC involves the development of aberrant crypt foci (ACF) as an intermediate step in the progression of cancer (3).

Generally CRC occurs in individuals older than 50 yr and develops as a consequence of environmental and/or genetic factors. Lifestyle factors including diet, overweight, low physical activity, alcohol intake, and smoking may account for 70% of CRC. Additionally, about 80% of all forms of sporadic CRC are characterized by one or more mutations in the adenomatous polyposis coli (APC) gene, of which about 60% result in the expression of a truncated version of the corresponding protein (4). The APC\textsuperscript{Min} mouse model used in current study is particularly advantageous in testing chemopreventive agents targeted against early stage lesions because adenomas grow in a grossly detectable size spontaneously within a few months. Epidemiologic, clinical, and laboratory animal studies have indicated that the type of dietary fat has been shown to modulate colon tumor development. Studies have shown that corn oil containing high levels of $\omega$-6 polyunsaturated fatty acid (5) enhances chemically induced carcinogenesis, whereas fish oil and mustard oil rich in $\omega$-3 polyunsaturated fatty acid (5,6) reduce azoxymethane (AOM)-induced colon tumor development in rats. Chemopreventive effects of dietary fish oil were found to be more effective in the postinitiation stage of colon carcinogenesis (7). The underlying mechanisms by which dietary fat composition exerts tumor enhancing or inhibiting effects has been under examination, and some mechanisms are beginning to emerge. For

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example, the chemopreventive effects of fish oil (8) and mustard oil (6) containing ω-3 fatty acids have been attributed to the inhibition of oxidative metabolism of arachidonic acid through the cyclooxygenase (COX) pathway (9,10). Overexpression of COX-2 in early stages of colon carcinogenesis and the inhibition of colon tumors by nonsteroidal anti-inflammatory drugs (NSAIDS) have suggested a tumor-promoting role for inflammatory prostaglandins in colon tumorigenesis (11–13). In another study, it was shown that rats given with AOM injections are fed ω-3 PUFA-containing diets had higher levels of apoptosis in colon cells compared with ω-6 PUFA-fed rats, possibly due to alteration in mitochondrial membrane composition and hence reactive oxygen species generation (14).

Recent studies from our laboratory have reported the chemopreventive effects of dietary flaxseed meal and flaxseed oil on AOM-induced colon tumor development (15,16). Flaxseed meal contains a high percentage of α-linolenic acid, an ω-3 fatty acid, and lignans. Lignans that mammals produce from dietary flaxseed meal have been shown to be protective against breast and colon cancer (17–19).

The purpose of the present investigation was to determine the chemopreventive effects of dietary flaxseed meal and flaxseed oil on intestinal tumor development in APCMin mice. Effects of dietary flaxseed meal and oil were also investigated on COX-1, COX-2, and apoptotic activities to elucidate the possible mechanism of action.

MATERIALS AND METHODS

Materials

Corn meal was prepared by grinding the yellow corn and flaxseed meal was prepared by grinding the omega variety of flaxseed provided by North Dakota Oilseed Council (Fargo, ND). AIN-93M was purchased from Dyets, Inc. (Bethlehem, PA). Corn oil was from Mazola, ACH Food companies, Inc. (Memphis, TN), and flaxseed oil was from Omega Nutrition (Bellingham, WA). Chloroform, ether, and tertiary-butyl ammonium hydroxide were purchased from Sigma Chemical Company (St. Louis, MO).

Animals

ApcMin mice (6 wk old) were purchased from Jackson Laboratory, Bar Harbor, Maine and divided into 5 groups of 8 each. Mice were placed on AIN-93M meal supplemented with either corn meal or flaxseed meal and corn oil and flaxseed oil.

Group assignments were as follows:

Group 1: Control (AIN-93M meal)
Group 2: Corn meal (AIN-93M, containing 15% corn meal)
Group 3: Flaxseed meal (AIN-93M, containing 15% flaxseed meal)
Group 4: Corn oil (AIN-93M, containing 15% corn oil)
Group 5: Flaxseed oil (AIN-93M, containing 15% flaxseed oil)

Supplemented diets were prepared by combining AIN-93M meal (850 g) with 150 g of corn meal or flaxseed meal, corn oil, or flaxseed oil in a mechanical mixer. Diets with corn meal were isocaloric with flaxseed meal, and diets with corn oil were isocaloric with flaxseed oil. Diets were stored in airtight containers at 4°C in a refrigerator. Peroxide content of the diets did not change during the storage period (20).

Mice were fed their respective diets and given water ad libitum throughout the duration of the experiment. Bowls filled with the respective diets were placed in the corresponding cages in the afternoon and replaced the following day. After 12 wk, mice were anesthetized with ether. Blood was collected by cardiac puncture. The gastrointestinal tract was removed and flushed thoroughly with ice-cold normal saline. The site, size, and number of tumors were recorded from entire gastrointestinal tract. Serum was prepared from blood by centrifugation. The serum samples, small intestine, and colon samples were analyzed for fatty acid composition and lignan levels. Colon samples were also used for COX-1, COX-2, and apoptotic studies.

Determination of Fatty Acids

The colon and intestinal samples collected from adjacent tumors from all mice in different groups were washed with 1.15% ice-cold KCl and blotted. The samples were minced and homogenized using an Omni GLH homogenizer (Omni International, Inc., Warrenton, VA). Samples of serum, colon, and intestine homogenates (160 µl) from mice combined with distilled water (1.6 ml) were extracted as fatty acid esters as described by us previously (15). Fatty acid analysis of the meals, serum, and colon samples was performed as described by Dwivedi et al. (15). A standard containing 32 individual fatty acids was used. Experimental samples may and do contain other fatty acids that are not present in our standard and therefore were not identified and reported as others. Most of these identified fatty acids were of 18 to 20 carbon and trans fatty acids. Results are reported as the percent mean values obtained from at least five individual samples.

Determination of Lignans

Lignan levels were determined in serum, colon, and intestinal homogenate suspension by using the same method as described by Bommareddy et al. (21).

Immunoblotting

Cyclooxygenase expression was determined by using the method described in our previous studies (16). Lysate proteins were resolved by sodium-dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane. The immunoreactive bands were quantitated by using a UVP Biochem Gel Documentation system (UVP, Inc., Upland, CA).
Determination of Apoptotic Cells on Colon and Small Intestinal Sections

To identify apoptosis on the colon and small intestinal samples, TACS TdT in situ apoptosis detection kit was used according to the procedures of manufacturer (R&D systems, Minneapolis, MN). Briefly, tissue samples from colon and small intestinal samples were first fixed to prevent the loss of low molecular weight DNA fragments. To make the DNA accessible to the labeling enzyme, the cell membranes were permeabilized with proteinase K reagent. Endogenous peroxidase activity was quenched using hydrogen peroxide. Next, biotinylated nucleotides were incorporated into the 3'-OH ends of the DNA fragments by terminal deoxynucleotidyl transferase. The biotinylated nucleotides were detected by using streptavidin-horseradish peroxidase conjugate followed by the substrate, diaminobenzidine (DAB). DAB-stained samples were examined using a light microscope. The enzyme reaction generated an insoluble colored precipitate where DNA fragmentation occurred.

Statistical Analysis

ANOVA followed by Student’s t-test was performed by INSTAT (GraphPad, San Diego, CA) software. A P value of <0.05 was considered significant.

RESULTS

Weight Gain

Body weights of the mice fed with various dietary meals are shown in Fig. 1. The body weights significantly decreased at the end of the experiment, which is mainly due to increased tumor burden in small intestine and decreased food consumption. None of the animals produced any visible toxicity or any gross changes in the internal organs.

Intestinal Tumor Data (Incidence, Multiplicity, and Size)

APC<sup>Min</sup> mice developed intestinal tumors (polyps), and most of these tumors appeared in the small intestine. These mice developed 15 to 28 tumors per mouse in the small intestine but only between 0 to 2 tumors per mouse in the colon. The tumor incidence, multiplicity, and size of polyps in small intestine (SI) and colon are summarized in Table 1. The tumor incidence was 100% in SI of all the groups. Dietary flaxseed meal and flaxseed oil significantly (P<0.05) suppressed intestinal polyp formation by about 45% compared with AIN, corn oil, and corn meal groups. There was no significant difference between tumor incidence among corn meal, corn oil, and AIN groups. As shown in Table 1, the tumor multiplicity in flaxseed meal and flaxseed oil groups was significantly (P<0.05) lower compared to corn meal and corn oil groups. The tumor size in dietary flaxseed meal and flaxseed oil groups was significantly (P<0.05) lower than control, corn meal, and corn oil in both small intestine SI and colon.

Fatty Acid Profile

The fatty acid compositions of dietary meals used in the current study are given in Table 2. The effects of dietary meal on serum fatty acid levels are given in Table 3. Levels of ω-6 fatty acids (linoleic and arachidonic acids) were significantly higher (P<0.05) in the serum of control, corn meal, and corn oil groups than in the flaxseed meal and flaxseed oil groups. Omega-3 fatty acid (α-linolenic acid, eicosapentaenoic acid,}

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of various diets on tumor development</td>
</tr>
<tr>
<td><strong>Small Intestine</strong></td>
</tr>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>AIN meal</td>
</tr>
<tr>
<td>Corn meal</td>
</tr>
<tr>
<td>Flaxseed meal</td>
</tr>
<tr>
<td>Corn oil</td>
</tr>
<tr>
<td>Flaxseed oil</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly lower (P<0.05) in flaxseed meal group than corn meal group.

<sup>b</sup>Significantly lower (P<0.05) in flaxseed oil than corn oil group.
### Table 2
Fatty acid composition of various dietary meals

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>AIN-93M</th>
<th>Corn Meal</th>
<th>Flaxseed Meal</th>
<th>Corn Oil</th>
<th>Flaxseed Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid 16:0</td>
<td>1.48</td>
<td>12.12</td>
<td>5.41</td>
<td>10.5</td>
<td>26</td>
</tr>
<tr>
<td>Palmitoleic acid 16:1</td>
<td>0.12</td>
<td>0</td>
<td>0.03</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>4.7</td>
<td>1.86</td>
<td>0.04</td>
<td>1.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>24.67</td>
<td>28.93</td>
<td>7.68</td>
<td>28.9</td>
<td>21.1</td>
</tr>
<tr>
<td>Linoleic acid (ω-6) 18:2</td>
<td>51.31</td>
<td>52.51</td>
<td>15.03</td>
<td>55.4</td>
<td>14.9</td>
</tr>
<tr>
<td>α-Linolenic acid (ω-3) 18:3</td>
<td>6.32</td>
<td>2.16</td>
<td>63.94</td>
<td>1</td>
<td>53</td>
</tr>
<tr>
<td>Others</td>
<td>2.4</td>
<td>2.48</td>
<td>7.80</td>
<td>22.0</td>
<td>0</td>
</tr>
</tbody>
</table>

*aData represent mean derived from at least 5 samples.

### Table 3
Effects of various diets on fatty acid composition of serum

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>AIN-93M</th>
<th>Corn Meal</th>
<th>Flaxseed Meal</th>
<th>Corn Oil</th>
<th>Flaxseed Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid 16:0</td>
<td>17.88</td>
<td>20.42</td>
<td>15.75</td>
<td>13.3</td>
<td>11.17</td>
</tr>
<tr>
<td>Palmitoleic acid 16:1</td>
<td>1.67</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>8.77</td>
<td>9.19</td>
<td>13.15</td>
<td>7.03</td>
<td>12.5</td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>21.57</td>
<td>22.69</td>
<td>16.37</td>
<td>23.37</td>
<td>15.22</td>
</tr>
<tr>
<td>Linoleic acid (ω-6) 18:2</td>
<td>35.83</td>
<td>32.1b</td>
<td>30.14</td>
<td>48.99c</td>
<td>29.34</td>
</tr>
<tr>
<td>α-Linolenic acid (ω-3) 18:3</td>
<td>3.49</td>
<td>2.75</td>
<td>18.08d</td>
<td>2.12</td>
<td>29.88e</td>
</tr>
<tr>
<td>Arachidonic acid 20:4</td>
<td>3.56</td>
<td>6.24</td>
<td>2.31</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (ω-3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Docosahexaenoic acid (ω-3)</td>
<td>22.6</td>
<td>3</td>
<td>4.2</td>
<td>1.21</td>
<td>1.86</td>
</tr>
<tr>
<td>Others</td>
<td>4.23</td>
<td>3.17</td>
<td>0</td>
<td>1.19</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*bSignificantly higher in corn meal group than flaxseed meal group (P < 0.05).

*cSignificantly higher in corn oil group than flaxseed oil group (P < 0.05).

*dSignificantly higher in flaxseed meal group than corn meal group (P < 0.05).

*eSignificantly higher in flaxseed oil group than corn oil group (P < 0.05).

### Table 4
Effects of various diets on fatty acid composition of small intestine

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>AIN-93M</th>
<th>Corn Meal</th>
<th>Flaxseed Meal</th>
<th>Corn Oil</th>
<th>Flaxseed Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid 16:0</td>
<td>28.49</td>
<td>25.56</td>
<td>14.9</td>
<td>14.22</td>
<td>12.25</td>
</tr>
<tr>
<td>Palmitoleic acid 16:1</td>
<td>16:1</td>
<td>0</td>
<td>3.16</td>
<td>0</td>
<td>1.02</td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>16.97</td>
<td>8.35</td>
<td>26.08</td>
<td>7.18</td>
<td>12.27</td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>20.86</td>
<td>24.31</td>
<td>15.42</td>
<td>25.98</td>
<td>23.33</td>
</tr>
<tr>
<td>Linoleic acid (ω-6) 18:2</td>
<td>10.31</td>
<td>10.6d</td>
<td>8.43</td>
<td>27.27c</td>
<td>17.99</td>
</tr>
<tr>
<td>α-Linolenic acid (ω-3) 18:3</td>
<td>0</td>
<td>1.25</td>
<td>3.4d</td>
<td>1.37</td>
<td>9.82e</td>
</tr>
<tr>
<td>Arachidonic acid 20:4</td>
<td>2.72</td>
<td>3.56</td>
<td>5.22</td>
<td>0.86</td>
<td>1.44</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (ω-3)</td>
<td>20.5</td>
<td>0</td>
<td>2.07</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Docosahexaenoic acid (ω-3)</td>
<td>22.6</td>
<td>4.21</td>
<td>6.35</td>
<td>0</td>
<td>2.77</td>
</tr>
<tr>
<td>Others</td>
<td>16.44</td>
<td>31.7</td>
<td>18.13</td>
<td>18.5</td>
<td>30.13</td>
</tr>
</tbody>
</table>

*aData represent mean derived from at least 5 samples.

*bSignificantly higher in corn meal group than flaxseed meal group (P < 0.05).

*cSignificantly higher in corn oil group than flaxseed oil group (P < 0.05).

*dSignificantly higher in flaxseed meal group than corn meal group (P < 0.05).

*eSignificantly higher in flaxseed oil group than corn oil group (P < 0.05).
TABLE 5
Effects of various diets on fatty acid composition of colon

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Ratio</th>
<th>AIN-93M</th>
<th>Corn Meal</th>
<th>Flaxseed Meal</th>
<th>Corn Oil</th>
<th>Flaxseed Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid 16:0</td>
<td>15.88</td>
<td>14.8</td>
<td>20.09</td>
<td>17.8</td>
<td>10.62</td>
<td></td>
</tr>
<tr>
<td>Palmitoleic acid 16:1</td>
<td>3.84</td>
<td>3.3</td>
<td>2.73</td>
<td>0</td>
<td>4.91</td>
<td></td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>11.23</td>
<td>5.4</td>
<td>10.86</td>
<td>14.06</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>22.36</td>
<td>30.3</td>
<td>16.4</td>
<td>21.2</td>
<td>33.4</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (ω-6) 18:2</td>
<td>16.4</td>
<td>29.61b</td>
<td>14.14</td>
<td>26.3</td>
<td>25.57</td>
<td></td>
</tr>
<tr>
<td>α-Linolenic acid (ω-3) 18:3</td>
<td>0</td>
<td>1.46</td>
<td>5.84c</td>
<td>0.8</td>
<td>15.21d</td>
<td></td>
</tr>
<tr>
<td>Arachidonic acid 20:4</td>
<td>3.91</td>
<td>3.06</td>
<td>3.5</td>
<td>7.58</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Eicosapentaenoic acid (ω-3) 20:5</td>
<td>0</td>
<td>0</td>
<td>1.38</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Docosahexaenoic acid (ω-3) 22:6</td>
<td>0.89</td>
<td>1</td>
<td>1.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>25.50</td>
<td>12.07</td>
<td>23.36</td>
<td>12.26</td>
<td>3.69</td>
<td></td>
</tr>
</tbody>
</table>

aData represent mean derived from at least 5 samples.

bSignificantly higher in corn meal group than flaxseed meal group (P < 0.05).
cSignificantly higher in flaxseed meal group than corn meal group (P < 0.05).
dSignificantly higher in flaxseed oil group than corn oil group (P < 0.05).

and docosahexaenoic acid) levels in the serum of flaxseed meal group and flaxseed oil group were significantly higher (P < 0.05), than that of other 3 groups.

The effects of dietary meal on small intestine fatty acid levels are given in Table 4. Levels of ω-6 fatty acids were significantly higher (P < 0.05) in control, corn meal, and corn oil groups than in the flaxseed meal, flaxseed oil groups. Omega-3 fatty acids levels of the flaxseed meal, flaxseed oil group were significantly higher (P < 0.05) than that of other three groups.

The fatty acid profile of different groups in the colon homogenates are given in Table 5. Levels of ω-6 fatty acids were significantly higher (P < 0.05) in the corn meal group than in the flaxseed meal group. Omega-3 fatty acids levels of the flaxseed meal group and flax oil group were significantly higher (P < 0.05) than that of other three groups.

Ratio of fatty acids (ω-3:ω-6) in serum, SI, and colon samples of all the groups is shown in Table 6. As can be seen from Table 6, the ratio of the fatty acids in the flaxseed meal and flaxseed oil group was relatively lower compared with other three groups.

Effects of Dietary Meals on Lignan Levels
Lignan levels in serum, small intestine and colon samples are given Table 7. Neither enterodiol nor enterolactone were detectable in the serum, small intestine and colon samples of the control group, corn meal or corn oil and flaxseed oil group. However, enterodiol and enterolactone were detected in the samples of flaxseed meal group.

Effects of Dietary Meals on COX-1 and COX-2 Expression
COX-1 and COX-2 expressions in colon samples are shown in Fig. 2. Both COX-1 and COX-2 levels were found to be

FIG. 2. Immunoblotting of expression of cyclooxygenase enzymes in colon samples. a, significantly lower (P < 0.05) in the flaxseed meal group than the corn meal group. b, significantly lower (P < 0.05) in the flaxseed oil group than the corn oil group.

TABLE 6
Effects of various diets on ratio of fatty acids in serum, small intestine (SI) and colon

<table>
<thead>
<tr>
<th>Group</th>
<th>Ratio of Fatty Acids (ω-3:ω-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>AIN meal</td>
<td>1:6.5</td>
</tr>
<tr>
<td>Corn meal</td>
<td>1:7.7</td>
</tr>
<tr>
<td>Flaxseed meal</td>
<td>1:1.6a</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1:15.6</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>1:1b</td>
</tr>
</tbody>
</table>

aSignificantly lower (P < 0.05) in flaxseed meal group than corn meal group.
bSignificantly lower (P < 0.05) in flaxseed oil than corn oil group.
Flax meal 0.42 ± Group EL ED EL ED EL ED
the corn meal, corn oil, and AIN treated groups and high levels
of ω-6 PUFA in the flaxseed treated groups indicates that the
type of fatty acid present in the biological samples depends on
the type of fatty acid incorporated in the diet.
The effects of dietary flaxseed meal on eicosanoid metabolism have been implicated as a potential mechanism
of an antipromoting effect on colon tumor development (23).

**Table 7**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum (µM) (Mean ± SE)</th>
<th>Colon (µmol/g) (Mean ± SE)</th>
<th>Small Intestine (µmol/g) (Mean ± SE)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EL</td>
<td>ED</td>
<td>EL</td>
</tr>
<tr>
<td>Serum</td>
<td>0.42 ± 0.016</td>
<td>0.07 ± 0.005</td>
<td>0.17 ± 0.093</td>
</tr>
<tr>
<td>Colon</td>
<td>0.05 ± 0.002</td>
<td>1.05 ± 0.002</td>
<td>0.14 ± 0.001</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.058 ± 0.007</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Abbreviations are as follows: EL, enterolactone; ED, enterodiol. Data represent mean derived from at least 5 samples.

significantly higher (*P* < 0.05) in the corn meal group when
compared to the flaxseed meal group. Similarly, COX-1 and
COX-2 were significantly higher (*P* < 0.05) in the corn oil
group as compared to the flaxseed oil group. The flaxseed meal
group had significantly lower (*P* < 0.05) COX-1 and COX-2
levels than the flaxseed oil group.

**Effects of Dietary Meals on Apoptosis**

TUNEL staining was used to localize apoptotic cells in the
colon and small intestine. There was no significant difference
on apoptotic cells among the all groups (data are not shown).

**DISCUSSION**

In the present study, dietary flaxseed oil and flaxseed meal
exhibited chemopreventive effects on intestinal tumor develop-
ment in APCMin mice. In this study, we showed that dietary
flaxseed meal and oil decreased intestinal tumor multiplicity
and average tumor size in the APCMin mouse model. The av-
erage number of tumors per mice in the small intestine and
colon was reduced by 40% and 18%, respectively, in the di-
etary flaxseed meal and flaxseed oil groups compared with
AIN-treated mice. Similarly, the reduction in small intestine
colon was 43% and 72%, respectively, when compared
with corn meal and corn oil treated groups. The average tu-
ror size in the corn meal, corn oil, and AIN groups was sig-
ificantly larger when compared with the flaxseed meal and
flaxseed oil group. These results show that tumors in dietary
flaxseed treated groups, besides being few, were also very small
in size when compared with corn meal, corn oil, and AIN treated
groups.

The fatty acids in serum, colon, and intestine are representa-
tive of fatty acids present in the diets given. This study showed
that the serum from the corn meal group had high levels of ω-6
fatty acids with very small amounts of ω-3 fatty acids, which
indicates that incorporation of ω-6 fatty acids in serum could
promote tumor promotion. The serum, small intestine and colon
of the flaxseed meal group had ω-3 and ω-6 fatty acids in a ratio
of 1:1.3, 1:1.6, and 1:1.2, respectively. The ratio of 1:3 of the ω-3
and ω-6 fatty acids is suggested to be optimal required for tu-
mor prevention (22). The groups fed with flaxseed meal and oil
provided the optimal amounts of both ω-3 and ω-6 fatty acids.
The high levels of ω-6 PUFA in the colon tissue homogenate of
the corn meal, corn oil, and AIN treated groups and high levels
of ω-3 PUFA in the flaxseed treated groups indicates that the
type of fatty acid present in the biological samples depends on
the type of fatty acid incorporated in the diet.

The effects of dietary flaxseed meal on eicosanoid metabolism have been implicated as a potential mechanism
of an antipromoting effect on colon tumor development (23).

The proinflammatory eicosanoids prostaglandin E2 (PGE2) and
leukotriene B4 (LTB4) are derived from the ω-6 fatty acid,
arachidonic acid, which is maintained at high cellular concentra-
tions by high ω-6 and low ω-3 polyunsaturated fatty acid content
of the modern Western diet (24). Decreased synthesis of one or
both of these eicosanoids has been observed after inclusion of
flaxseed oil or fish oil in the diet (24). Omega-3 fatty acids
present in a dietary flaxseed meal inhibit oxidative metabolism
of arachidonic acid by the cyclooxygenase pathway responsible
for prostaglandin synthesis (25). Studies have indicated that
prostaglandin synthesis inhibitors prevent colon carcinogenesis
(26). Two forms of COX have been identified. COX-1 expres-
sion is constitutive in most tissues including the gastrointestinal
tract; whereas COX-2 mRNA and protein are highly inducible
by inflammatory and growth factors. COX-2 mRNA and protein
are significantly elevated in the human colon tumor. COX-1 expres-
sion remained the same or decreased (27,28). In this study,
arachidonic acid levels in the serum and colon samples of corn
and flaxseed meal treated groups indicated that more than one
third of linoleic acid in the diet is converted to arachidonic acid.

Higher multiplicity of intestinal tumors, high levels of arachi-
donic acid, and high levels of COX-1 and COX-2 expres-
sion in the colon samples of the corn meal, corn oil and
AIN groups were observed when compared to flaxseed meal
and oil groups. The possible mechanism for enhanced tu-
mor development in the corn meal, corn oil, and AIN groups
may have been due to the metabolism of arachidonic acid to
prostaglandins, which act as tumor promoters. Conversion of
arachidonic acid to prostaglandins is mediated by cyclooxy-
genase. High levels of this enzyme in the corn meal group
may have resulted in enhanced tumor development. In the
flaxseed groups, ω-3 fatty acids may have been metabolized
to trienoic series of prostaglandins and 5-series of leukotrienes.
These have anti-inflammatory properties and therefore would
have decreased tumor multiplicity and size (22). Furthermore,
high levels of α-linolenic acid may have inhibited desaturases
and elongase, thus decreasing the synthesis of arachidonic
acid.
A common characteristic of tumor cells is that they lack the ability to carry out apoptosis (29). Studies have shown that mammalian lignans suppressed colo 201 human colon cancer cell growth both in vitro and in vivo through apoptosis and decreased cell proliferation (19). In the present study, the number of apoptotic cells was similar among control, corn oil and meal, and flaxseed oil and meal treated groups. Thus, ω-3 fatty acids and/or lignans may not be inducing apoptosis at the concentration available in tissues under our experimental conditions. Recent preliminary experiments (unpublished observations) on the effects of enterodiol and enterolactone in CACO-2 cells have indicated the involvement of Wnt and β-catenin signaling as possible mechanisms of action.

Several experimental studies have shown that dietary oils (fish, mustard, flaxseed, and perilla) containing ω-3 fatty acids reduce colon tumor development (6–9,15,28). Flaxseed has been shown to reduce colon cancer markers such as the number of aberrant crypts and aberrant crypt foci (ACF) in short term and size and multiplicity of ACF over long term in animal feeding studies (30). Lignans extracted from flaxseed also decreased ACF multiplicity (17). In a study involving both full-fat or defatted flaxseed meal, it was observed that both had similar effects on markers for colon cancer. These findings suggest that the effects observed were not due to the oil but could be best ascribed to flax lignan (17). A pilot study of Demark-Wahnefried et al. (31) suggested that a flaxseed-supplemented, fat-restricted diet may affect the biology of the prostate and associated biomarkers. Dietary flaxseed inhibited the growth of human estrogen-dependent breast cancer and strengthened the tumor inhibitory effect of tamoxifen (17). Dietary flaxseed reduced the growth and metastasis of estrogen receptor negative human breast cancer in part due to its lignan and oil components (32). Dietary flaxseed also inhibited tumor metastasis after surgical excision of the primary tumor (33). Van Kranen et al. (2) showed that diet supplemented with 5% flaxseed or 30% rye bran did not provide any beneficial effect on the onset and progression of intestinal neoplasia in ApcMin mice. However, in the present study, we showed that 15% flaxseed meal containing α-linolenic acid, an ω-3 fatty acid (60%), and lignans provided a high degree of chemoprevention against intestinal tumor development in mice. This could have been possible by both adjusting to the optimal ratio of ω-6 to ω-3 fatty acids and the chemopreventive effects of lignans (19,20).

Among the various risk factors, diet and nutrition have been implicated as important variables associated with colon carcinogenesis (34). The extended time duration in the conversion of adenoma to adenocarcinoma provides a window of opportunity for dietary intervention (35). Experimental studies have shown that diets high in red meat and animal fat were associated with an increased risk of colorectal cancer (36). High-fat diets containing corn oil, safflower oil, beef fat, or lard increased chemically induced colon tumors in laboratory animals as compared to low fat diets containing high levels of coconut oil, olive oil, or trans-fat did not exhibit tumor-enhancing effects. Fatty acid composition of a high-fat diet is critical in modulating the tumor outcome and not the total amount of fat for the development of colon cancer (37).

The chemopreventive effects of dietary flaxseed in the APCMin mouse model have potential implications for adenoma regression in human familial adenomatous polyposis (FAP), which is also caused by a germline mutation of APC (38). However, limitations of this model reflects the effects on only inherited types of tumorigenesis and not sporadic colorectal carcinogenesis and more small intestine polyps in contrast to colon polyps in humans (39–41).

Dietary flaxseed oil and meal are an effective chemopreventive agent against colon and intestinal tumor development in experimental animal models, and its chemopreventive effects are attributed to the high levels of ω-3 PUFAs and lignans when compared to the control diet with high levels of ω-6 PUFAs. Further studies are needed to establish the optimal amount of flaxseed that should be incorporated into the diet and elucidate the possible mechanism of action that dietary flaxseed has on colon cancer chemoprevention.

REFERENCES


