Preventive Potential of Wheat Bran Fractions against Experimental Colon Carcinogenesis: Implications for Human Colon Cancer Prevention


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ABSTRACT

Epidemiological studies suggest an inverse relationship between the intake of dietary fiber, particularly fiber from cereal grains, and colon cancer risk. Animal model assays have demonstrated that the protective effects of dietary fiber on colon cancer development depend on the nature and source of the fiber. Wheat bran (WB) appears to inhibit colon tumorigenesis more consistently than do oat bran or corn bran. This study was designed to determine whether specific WB fractions such as WB fiber, WB lipids, or phytytic acid differentially affect colon carcinogenesis in a well-established colon cancer model. In addition, the modulating effect of specific fractions of WB on the activities of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-1 and COX-2 enzymes were assessed in colon tumors as those have been shown to play a role in tumor progression. At 5 weeks of age, groups of male F344 rats were assigned to one of six diets: a high-fat diet containing 10% WB (control diet) and experimental high-fat diets containing 10% defatted WB (WB-P), 10% defatted WB (WB-F), 10% defatted and defatted WB (WB-PF), 10% WB-PF fortified with 2% bran oil and/or with 0.4% phytylate. At 7 weeks of age, all rats except those in the vehicle-treated groups were given two weekly s.c. injections of azoxymethane (AOM) at a dose rate of 15 mg/kg body weight/week. They continued to receive their respective diets until 50 weeks after carcinogen treatment and were then killed. Colon tumors were analyzed for iNOS, COX-1, and COX-2 expression and enzymatic activities. Colon tumors were evaluated histopathologically and classified as adenomas and adenocarcinomas. We found that removal of phytic acid (WB-P) or lipids (WB-F) from WB had no significant effect on colon tumor incidence (% animals with tumors) or multiplicity (tumors/animal), whereas removal of both phytate and lipids from WB (WB-PF) significantly increased colon tumor multiplicity and volume. Interestingly, WB-PF fortified with excess bran oil or with bran oil plus phytylate significantly inhibited colon tumor incidence, multiplicity, and volume; but supplementation of WB-PF with phytylate alone had no significant effect on colon tumorigenesis in rats suggesting that lipid fraction of WB possesses tumor-inhibitory properties. Moreover, feeding WB-PF diet significantly increased iNOS, total COX and COX-2 enzyme activities, and INOS protein expression in colon tumors as compared with wheat bran control diet. Feeding the WB-PF that was fortified with excess bran oil alone or with bran oil plus phytylate significantly suppressed the activities of iNOS and COX-2 as well as the expression of INOS and COX-2 in colon tumors compared with that in rats fed the WB diet or WB-PF diet. The study demonstrates for the first time that the lipid fraction of wheat bran has strong colon tumor inhibitor properties. The exact mechanism(s) by which the lipid fraction of WB inhibits colon carcinogenesis in addition to alteration of INOS and COX activities remains to be elucidated. Additional studies are warranted to identify biologically active constituents of lipid fraction of WB and their relative role in colon tumor inhibition.

INTRODUCTION

Cancer of the colon is a major neoplastic disease affecting men and women in Western countries including the United States and Canada (1). Since Burkitt’s pioneering research that pointed to inverse relationships between colon cancer risk and consumption of fiber-rich foods, many epidemiological and laboratory animal studies have tested this hypothesis (2, 3). Intracountry comparisons of dietary fiber and colon cancer incidence and mortality rates support the hypothesis that dietary fiber, especially fiber from cereal sources, protects against colon cancer (4–6). Prospective studies have been only somewhat supportive in this regard in that they have shown either a protective association or no association (7). Case-control studies on the relationship between dietary fiber and colon cancer provided convincing results. Howe et al. (8) and Trock et al. (9) independently performed meta-analyses of several case-control studies conducted in populations with different colon cancer rates and dietary practices. They showed that relative risk decreased significantly as the fiber intake increased. Freudenheim et al. (10) found that colon cancer risk decreased with increased intake of grain fiber and that insoluble grain fiber was more strongly associated with this risk reduction than the soluble fiber from vegetables and fruits. Studies in metabolic epidemiology demonstrated that populations who are at low risk for colon cancer and consume diets high in dietary fiber and/or low in dietary fat excrete low levels of putative colon tumor promoters such as secondary bile acids compared with those at high risk for colon cancer who consume diets with low fiber content and/or high in fat content (4, 11, 12). Diet intervention studies in humans consuming a high-fat/low-fiber diet have also demonstrated that adding WB (13) to the diet favorably altered a number of biomarkers related to colon cancer risk including fecal mutagenicity (13) and secondary bile acids (14). In such studies, WB proved to be more effective than corn bran or oat bran, suggesting that the modifying effect of dietary fiber on the production of putative tumor promoters depends on the type of fiber consumed. Laboratory animal model studies have corroborated this and indicated that protective effects of dietary fiber in colon carcinogenesis depend on the type of fiber and that WB but neither corn bran nor oat bran appears to inhibit colon tumor development more consistently (15–19).

The reasons for the protective effect of dietary WB against colon tumor development have not been fully explored. Diet intervention studies in humans indicate that WB supplementation increases the fecal bulk, thereby diluting potential carcinogens and tumor promoters in the lumen of the colon and decreasing the formation of colonic mutagens and secondary bile acids (13, 14). Animal model studies also demonstrate that, of all of the fibers evaluated, WB is the best diluter of colonic contents (18). Furthermore, WB is a rich source of phytochemicals, such as phytate, and of lipid-soluble components, such as phytosterols and tocopherols, to cite a few. Among the components of dietary fiber, especially WB, phytic acid has been studied extensively for its potential chemopreventive activity against colon carcinogenesis (20, 21). Indeed, phytic acid administered in drinking water has been shown to inhibit colon tumorigenesis (20, 21). However, the role of lipids and lipid-soluble components that are present in WB has not yet been explored in experimental colon carcinogenesis. The present study was therefore designed as an initial...
step to evaluate the inhibitory activity of specific fractions of WB against colon carcinogenesis in the F344 rat model. For this study, WB-P and/or WB-F were compared with intact WB. In addition, WB oil (lipid fraction) and/or phytate in excess were added back to the WB-PF and compared with WB-P, WB-F, or WB-PF and with intact WB to assess the specific colon tumor-inhibitory properties of phytate and wheat bran oil supplemented in excess in the diet.

The nature of the chemical constituents of WB oil has not been fully delineated. It is known that bran oil is a rich source of fatty acids and several lipid-soluble substances, such as phytosterols, tocopherols, and phenolic compounds, possessing antioxidant and/or anti-inflammatory properties. Some of the mechanisms by which antioxidants and anti-inflammatory agents inhibit tumorigenesis involve the modulation of iNOS, a distinct, Ca$^{2+}$-independent isoform of NOS (22) and of COX isoforms, which are rate-limiting enzymes catalyzing the conversion of AA into eicosanoids (23, 24). COX-2 has been characterized as an early response gene that is rapidly induced after stimulation of quiescent cells by mitogenic stimuli (24). Studies in our laboratory and elsewhere have demonstrated that colonic tumors of laboratory animals and humans have increased expression and/or activities of iNOS and COX-2 when compared with levels in adjacent normal mucosa (22, 25, 26). Tsujii and DuBois (24) have reported that intestinal epithelial cells overexpressing the COX-2 gene develop altered adhesion properties and resist apoptosis. iNOS has been shown to be involved in the regulation of COX-2 (27). Taken together, these observations imply that iNOS and COX play a critical role in colon tumor growth and progression. In the present study, we tested the hypothesis that colon tumor modulation by WB components, specifically the lipid fraction, may be mediated, in part, through the changes in the activities and expression of iNOS and COX in colonic tumors.

MATERIALS AND METHODS

Materials

AOM (CAS: 25843-45-2) was purchased from Ash Stevens (Detroit, MI). WB, WB-P, WB-F, WB-PF, and WB oil were kindly supplied by Kellogg Co. (Battle Creek, MI). The fractionation of WB-P from WB provided by Kellogg Co. was as follows. WB was extracted with 0.5M HCl (1:20, bran:HCl), agitated for 60 min, and then filtered. The sediment was then washed with demineralized soft water, pressed to remove water, and dried under forced air at 70°C. The WB-F fraction was prepared by extracting WB with hexane at 50°C and filtering the lipid fraction, and the resulting WB-F fraction was dried. WB oil fraction was prepared by extracting WB with hexane at 50°C. The extract was subjected to repeated filtration, and the hexane/oil mixture was desolventized at 60°C and purified. The fat content of WB-F fraction was 0.90%, indicating that 99.1% fat has been extracted from WB. WB-F was used to prepare the WB-PF fraction. WB-F was extracted with 0.5 m HCl as described above for the fractionation of WB-P. The resulting WB-PF fraction was washed with soft water, pressed to remove water, and dried under forced air at 70°C as described above. Phytate (phytic acid dodeca sodium salt) derived from rice was purchased from Sigma Co. (St. Louis, MO) and mixed in an equal amount with phytin, a natural phytate derived from rice kindly provided by Tsuno Rice Fine Chem. Co. Ltd. (Wakeyama, Japan). Rabbit polyclonal antibody of iNOS was obtained from Cayman Chemicals (Ann Arbor, MI), and goat polyclonal antibodies of COX-1 and COX-2 were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). iNOS, COX-1, and COX-2 purified proteins were purchased from Cayman Chemicals (Ann Arbor, MI). The ECL chemiluminescence system was obtained from Amersham Life Science, Inc. (Arlington Heights, IL).

Animals and Diets

Weanling male F344 rats were received from the Charles River Breeding Laboratories (Kingston, NY). All ingredients of the semipurified diet were bought from Dyets, Inc. (Bethlehem, PA) and stored at 4°C prior to preparation of experimental diets. The compositions of the control diet containing high fat and WB bran and of experimental diets containing high fat and different portions of WB were shown in Table 1. The formulations of all experimental diets containing WB and different WB fractions were based on the AIN-76A diet, which contained 5% alphacel as a source of fiber (15–17). All experimental diets in the current study contained 5% alphacel. Control diet and experimental diets containing different fractions of WB were prepared weekly in our laboratories and were stored in a cold room. The proximate analysis of WB oil was as follows: total lipids, 91.5%; total fat, 70.6%; total phytosterols, 1802 mg/100 g, (campesterol, 392 mg/100 g; stigmasterol, 90 mg/100 g; and β-sitosterol, 1320 mg/100 g); and phytic acid, 0. The major fatty acids present in the WB oil are oleic acid, palmitic acid, and linoleic acid. The phytic acid levels of WB and WB-F were 4.7 and 4.5 g/100 g, respectively. The fat content of WB was about 2.77%. The levels of phytate and WB oil added to WB-P, WB-F, and/or WB-PF were 0.4 and 2.0%, respectively (Table 1). The amount of sodium contributed by the phytate mixture added to the diets was about 475 ppm. The rationale for choosing 2% bran oil as a supplement was to determine the protective effect of lipid fraction against colon carcinogenesis when given in excess because it contains several compounds with potential chemopreventive properties. Because excess phytic acid in the diet has been suspected to induce mineral imbalances, phytic acid was added to an experimental diet at the 0.4% level.

Experimental Procedure

Efficacy Study. A total of 252 male F344 rats received at weaning were quarantined for 1 week and had free access to the high-fat diet containing 10% WB (control diet). After quarantine, all rats were randomly distributed so that the body weights in each group were evenly distributed (30 rats for each AOM-treated group and 6 rats for each saline-treated group). Beginning at 5 weeks of age, the rats had access to their respective control diet and experimental diets containing 10% WB-P, WB-F, or WB-PF and WB-PF supplemented with 0.4% phytate and/or 2% wheat bran oil (Fig. 1 and Table 1). At 7 weeks of age, the rats scheduled to receive carcinogen treatment were s.c. injected with a solution of AOM at a dose rate of 15 mg/kg body weight, once weekly for 2 successive weeks. Rats intended for vehicle treatment received an

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Table 1 Percentage composition of high-fat experimental diets containing WB and its fractions

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>WB</th>
<th>WB-P</th>
<th>WB-F</th>
<th>WB-PF</th>
<th>WB-PF plus phytate</th>
<th>WB-PF plus bran oil</th>
<th>WB-PF plus phytate and bran oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
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<td>Corn starch</td>
<td>31.48</td>
<td>31.48</td>
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<td>31.48</td>
<td>31.08</td>
<td>31.48</td>
<td>31.08</td>
</tr>
<tr>
<td>Dextrose</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Mineral Mix (AIN-76A)</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Wheat bran supplement</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
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<tr>
<td>Phytate</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WB oil</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Experimental diets contain 10% of each WB, WB-P, WB-F, or WB-PF. WB-PF plus phytate and WB-PF plus bran oil contain 0.4% phytate and 2% WB oil, respectively, whereas WB-PF plus phytate and bran oil contain 0.4% phytate and 2% WB oil in addition to WB-PF supplement.
equal volume of normal saline. All dietary regimens were continued until 50 weeks after the second AOM treatment. Body weights were recorded every week for the first 10 weeks and then every 4–6 weeks. Dying or moribund rats were killed and necropsied. At the termination of the experiment, all rats were killed by asphyxiation with CO2 and were necropsied. Colon tumors were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by routine histological methods with H&E staining. The sections were examined for tumor types according to the classification that is routinely used in our laboratory (28).

Adenocarcinomas of the colon were malignant tumors that have the tendency to form adenomatous structures. Adenomas were benign tumors that formed abnormal glandular structures with less atypism.

Biochemical Analysis

Sample preparation for analyses of iNOS and COX activities and/or expression was as described previously (22, 28). Samples of colonic tumors were homogenized in 1:3 (w/v) volumes of homogenizing buffer containing 30 m M of 20 m M HEPES, and they were loaded onto a Dowex AG 50W-X8 resin column. L-[3H]Citrulline was eluted and separated on TLC. Radioactivity was counted by a BioScan Radiomatic detector. Results are expressed as pmol L-[3H]citrulline released/mg protein/20 min.

Total COX and COX-2 Synthetic Activity. COX activities in colon tumors were assayed by using a slight modification of a method published previously (29). The microsomal pellet was resuspended in 50 mm potassium phosphate buffer (pH 7.4) for assay of total COX and COX-2 activities. For determining total COX activity, 150 m M reaction mixture containing 12 m M[14C]AA (420,000 dpm), 1 m M epinephrine, 1 m M glutathione in 50 m M phosphate buffer, and 25–35 mg of tumor microsomal protein were incubated at 37°C for 15 min. For determining COX-2 activity, the reaction mixture was preincubated with 150 m M of aspirin to block COX-1 activity and to modify COX-2 activity. After incubation, the reaction was terminated by adding 40 m M of 0.2 m HCl. The COX metabolites of AA were extracted three times with 0.5 ml of ethyl acetate. The combined extracts were evaporated to dryness under N2 and redissolved in chloroform and subjected to TLC on Silica G plates. The TLC plates were developed in a solvent system containing chloroform: methanol:acetic acid:water (100:15:1:25,v/v/v/v) and were exposed in an iodide chamber for 5 min to visualize the standards. The metabolites of [14C]AA corresponding to PG2, PGI2, PGD2, 6-keto-PGF1α, and TXB2 were detected by their comigration (Rf) with authentic standards for total COX activity and [14C]-15(R)-hydroxyeicosatetraenoic acid for COX-2 activity.

Western Blot Analyses of iNOS, COX-1, and COX-2. iNOS, COX-1, and COX-2 purified proteins, purchased from Cayman Chemicals, were used as electrophoresis standards. The proteins were separated on an 8% PAGE gel and then electroblotted on polyvinylidene difluoride membranes as described (30). After blocking membranes in 5% nonfat dry milk, they were incubated with antibodies of COX-1, COX-2, and iNOS for 1 h. The membranes were washed three times and incubated once more with secondary horseradish peroxidase-linked antigen or antirabbit IgG antibody at a final concentration of 1:2000. The membranes were developed by the ECL chemiluminescence system and exposed to Kodak XAR5 film. Intensities of each band were scanned by a computing densitometer.

Table 2

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Tumor incidence (% animals with adenomas and/or adenocarcinomas)</th>
<th>Tumor multiplicity (tumors/animal)</th>
<th>Tumor volume (mm3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenomasa</td>
<td>Adenocarcinomasa</td>
<td>Totalc,d</td>
</tr>
<tr>
<td>Control, 10% WB</td>
<td>17 (1)</td>
<td>77 (23)</td>
<td>77 (23)</td>
</tr>
<tr>
<td>WB-P, 10%</td>
<td>3 (1)</td>
<td>67 (20)</td>
<td>70 (21)</td>
</tr>
<tr>
<td>WB-F, 10%</td>
<td>0</td>
<td>70 (21)</td>
<td>70 (21)</td>
</tr>
<tr>
<td>WB-PF, 10%</td>
<td>7 (2)</td>
<td>87 (26)</td>
<td>94 (26)</td>
</tr>
<tr>
<td>WB-PF plus 0.4% phyte</td>
<td>7 (2)</td>
<td>70 (21)</td>
<td>70 (21)</td>
</tr>
<tr>
<td>WB-PF plus 2% bran oil</td>
<td>0</td>
<td>30 (9)</td>
<td>30 (9)</td>
</tr>
<tr>
<td>WB-PF plus 0.4% phyte and 2% WB oil</td>
<td>0</td>
<td>43 (13)</td>
<td>43 (13)</td>
</tr>
</tbody>
</table>

a Number of colon tumors is given in parentheses.

b Total tumors include both adenomas and adenocarcinomas. Some rats had both adenomas and adenocarcinomas in the colon, whereas some of them had either adenomas or adenocarcinomas.

c Mean ± SD.

d Values in parentheses indicate percentage of increase (+) and decrease (−) in tumor volume in animals fed the WB diet.

Significantly different from 10% WB group (P < 0.03), WB-P group (P < 0.01), and WB-P group (P < 0.05) by Welch’s t test.

Significantly different from 10% WB group, WB-P group, WB-F group, and WB-PF group by Fisher’s exact probability test: P < 0.0007; P < 0.02.

Significantly different from 10% WB group (P < 0.005), WB-B group (P < 0.005), WB-PF group (P < 0.002), WB-PF group (P < 0.001), and WB-PF plus phytate group (P < 0.005).

Significantly different from WB-P group (P < 0.002), WB-PF group (P < 0.007), and WB-PF plus phytate group (P < 0.007) by Welch’s t test.

Significantly different from 10% WB group (P < 0.02 to P < 0.01), WB-F group (P < 0.03), WB-PF group (P < 0.001), and WB-PF plus phytate group (P < 0.005) by Welch’s t test.

Significantly different from defatted WB-F group (P < 0.03), WB-PF group (P < 0.04), and WB-PF plus phytate (P < 0.04) by Welch’s t test.

4794
PREVENTION OF COLON CANCER BY WHEAT BRAN FRACTIONS

Table 3: Modulation of iNOS and COX activities in colonic tumors by dietary WB fractions in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>iNOS activity</th>
<th>Total COX activity</th>
<th>COX-2 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB, 10%</td>
<td>208 ± 22**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB-PF, 10%</td>
<td>312 ± 30*</td>
<td>626 ± 74*</td>
<td>394 ± 37*</td>
</tr>
<tr>
<td>WB-PF plus 2% WB oil</td>
<td>126 ± 11**/i</td>
<td>274 ± 35**/i</td>
<td>143 ± 16**/i</td>
</tr>
<tr>
<td>WB-PF plus 2% WB oil and 0.4% phytate</td>
<td>142 ± 13**/i</td>
<td>347 ± 32**/i</td>
<td>205 ± 22**/i</td>
</tr>
</tbody>
</table>

* iNOS activity is expressed as pmol [3H]citrulline formed/mg protein/20 min.
** Total COX activity is expressed as pmol of [3H]AA metabolized/mg protein/15 min.
\( \beta \) COX-2 activity is expressed as pmol of [3H]6-hydroxy-2E2,4E5,8E9Z-eicosatetraenoic acid formed/mg protein/15 min.
\( \alpha \) Mean ± SE (n = 4–6 tumors).
\( \gamma \) Significantly increased from 10% WB diet group by Welch’s t test, \( P < 0.01 \).
\( \delta \) Significantly decreased from 10% WB diet group or WB-PF diet group by Welch’s t test, \( P < 0.01 \) to \( P < 0.001 \).
\( \varepsilon \) Significantly decreased from WB-PF diet group by Welch’s t test, \( P < 0.01 \).

Statistical Analysis

Body weights, colon tumor incidence (percentage of animals with tumors), multiplicity (mean number of tumors/animal), and tumor volume were determined for all rats fed the control diet and for those given experimental diets containing different fractions of WB. Body weights, tumor multiplicity, and tumor volume were analyzed and compared by Welch’s t test. Tumor incidence was analyzed by Fisher’s exact probability test. The differences were considered statistically significant at \( P < 0.05 \).

RESULTS

The body weights of rats treated with AOM or saline and fed the control or experimental diets containing fractions of WB were comparable throughout the study (data not shown; \( P > 0.05 \)). The results, summarized in Table 2, indicate that, in AOM-treated rats given the WB control diet or experimental diets containing different fractions of WB, >93% of the colon tumors were adenocarcinomas, and the rest were adenomas. None of the saline-treated animals on control or experimental diets developed colon tumors (data not shown in the table). WB-P or WB-F had no significant effect on the incidence and multiplicity of colon adenocarcinomas compared with effects of the WB control diet, suggesting that removal of phyto- or bran oil (lipid fraction) from WB did not have any significant effect on colon tumorigenesis. Interestingly, the WB-PF diet significantly increased the multiplicity of colon tumors (35% increase; \( P < 0.03 \)) compared with the WB control diet, indicating that removal of both phytate and bran oil, which are bioactive components of WB, produced a synergistic effect in enhancing colon tumorigenesis. It is noteworthy that fortification of WB-PF with 0.4% phytate had no inhibitory effect on colon tumorigenesis. However; WB-PF supplemented with 2% bran oil alone or 0.4% phytate plus 2% bran oil significantly suppressed the incidence of adenocarcinomas of the colon (44–61% inhibition; \( P < 0.02 \) to \( P < 0.001 \)) as compared with that of WB, WB-P, WB-F, or WB-PF. In addition, fortification of WB-PF with bran oil or phytate plus bran oil significantly inhibited multiplicity (45–63% inhibition; \( P < 0.0003 \) to \( P < 0.0001 \)) of colon adenocarcinomas compared with WB diet, WB-P, WB-F, WB-PF, or WB-PF plus phytate diet. This suggests that WB oil is one of the major components of WB that has inhibitory activity against colon carcinogenesis. It should be noted that although WB oil inhibits colon carcinogenesis, WB oil intake was approximately 4–5-fold greater in WB oil-fortified treatments than in the WB group. Results summarized in Table 2 also demonstrate that colon tumor volume was significantly reduced in animals fed WB-PF fortified with 2% WB oil alone or with 0.4% phytate plus 2% bran oil as compared with tumor volume in rats fed WB-PF (42–58% inhibition; \( P < 0.03 \) to \( P < 0.01 \)). Although the differences did not reach a statistical significance, diets containing WB-F or WB-PF but not WB-P increased the colon tumor volume by about 38–43% compared with tumor volumes in rats fed the WB control diet. Colon tumor volume data corroborate the results of colon tumor incidence and multiplicity and further indicate that WB oil is one of the major components of WB that has colon tumor inhibitory properties.

We also investigated whether the inhibition of colon carcinogenesis by the lipid fraction is associated with the modulation of iNOS and COX activities in the colon tumors. Results summarized in Table 3 indicate that dietary WB-PF significantly increased the activities of iNOS and total COX and COX-2 in the colonic tumors (\( P < 0.01 \)), whereas WB-PF plus 2% wheat bran oil in the diet significantly inhibited these enzyme activities as compared with that in rats fed WB diet (\( P < 0.01 \)). Interestingly, iNOS and total COX and COX-2 activities were significantly inhibited in colonic tumors of rats receiving the WB-PF diet supplemented with 2% bran oil alone or with a composite of 2% bran oil and 0.4% phytate compared with these activities in rats fed the WB-PF diet (\( P < 0.01 \)), suggesting that the inhibitory activity of WB fractions on these enzymes lies mainly in the lipid fraction.

A representative immunoblot analysis of iNOS, COX-1, and COX-2 expressions in colon tumors of animals on different dietary regimens is shown in Fig. 2. iNOS, COX-1, and COX-2 bands comigrated with their respective purified standards (Fig. 2, Lane 1). A standard curve of integrated absorbance from the laser densitometric scans of iNOS, COX-1, and COX-2 standards was obtained to quantitate immunoreactive iNOS, COX-1, and COX-2 proteins, and the results are summarized in Table 4. The results also indicate that the modulation of iNOS and COX isoforms protein expressions by WB oil, and phytate was the same as observed for their enzyme activities. Removal of phytate and lipids from WB (WB-PF) significantly increased iNOS expression in the colonic tumors as compared with that of rats fed the WB diet (\( P < 0.01 \)). Dietary WB-PF plus 2% bran oil

[Image 308x232 to 559x374]

Fig. 2. Representative immunoblot analysis of COX-1, COX-2, and iNOS expressions in colon tumors of animals fed diets containing WB and its fractions. Lane 1, COX-1, COX-2, and iNOS Western blot standards. Lane 2, colon tumors of animals fed WB. Lane 3, colon tumors of animals fed WB-PF. Lane 4, colon tumors of animals fed WB-PF plus 2% bran oil. Lane 5, colon tumors of animals fed WB-PF plus 2% bran oil and 0.4% phytate. \( kd \), molecular weight in thousands.

Table 4: Modulation of iNOS and COX-1 and COX-2 protein expression in colonic tumors by dietary WB fractions in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>iNOS activity</th>
<th>COX-1 activity</th>
<th>COX-2 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB, 10%</td>
<td>128 ± 14</td>
<td>72 ± 9.4</td>
<td>84 ± 9.2</td>
</tr>
<tr>
<td>WB-PF, 10%</td>
<td>184 ± 21*</td>
<td>88 ± 10</td>
<td>93 ± 11</td>
</tr>
<tr>
<td>WB-PF plus 2% WB oil</td>
<td>39 ± 6**</td>
<td>64 ± 6.4</td>
<td>36 ± 5.3**</td>
</tr>
<tr>
<td>WB-PF plus 2% WB oil and 0.4% phytate</td>
<td>66 ± 8.8*</td>
<td>74 ± 8.3</td>
<td>45 ± 6.7*</td>
</tr>
</tbody>
</table>

* Expression is expressed as ng of iNOS and COX-1 and COX-2/mg protein.
** Mean ± SE (n = 4–6 tumors).
\( \gamma \) Significantly increased from the 10% WB diet group by Welch’s t test, \( P < 0.01 \).
\( \delta \) Significantly decreased from the 10% WB diet group and WB-PF diet group by Welch’s t test, \( P < 0.01 \).
alone or 0.4% phytate and 2% bran oil significantly suppressed the expressions of iNOS and COX-2 in the colonic tumors compared with tumors in rats given the WB diet or WB-PF diet \((P < 0.001\) to \(P < 0.0001\)). These diets had no significant effect on the constitutive COX-1 expression.

**DISCUSSION**

Experimental studies provide evidence that of all dietary fibers evaluated, WB affords the most protection against colon carcinogenesis (15–19). WB is comprised of a heterogeneous group of nonstarch polysaccharides, noncarbohydrate substances such as lignin, phytate, and lipids, as well as lipid-soluble substances. One will need to evaluate bioactive components in the WB to elucidate which of these are responsible for the observed protective effect. The major purpose of this study was therefore to begin identifying the active components in WB that reflect its inhibitory properties against colon carcinogenesis.

The outcome of this study is of great interest because of its implication for human colon cancer prevention. The present study demonstrates for the first time that removal of lipids and lipid-soluble components from WB increased colon tumorigenesis, whereas fortification of WB-PF diet with bran oil significantly inhibited colon tumorigenesis. This suggests that the lipid fraction of WB contains bioactive agents that inhibit colon carcinogenesis. It is also noteworthy that the degree of inhibition of colon carcinogenesis in the rats fed the bran oil-fortified diet exceeds that seen with WB diet, which we had evaluated previously for its colon tumor-inhibitory activity in a similar experimental design (15). However, in the current study, WB oil intake in animals fed WB-PF fortified with bran oil was ~4-fold higher than in those fed the WB control diet, suggesting that bioactive compounds present in the WB oil possess inhibitory properties against colon carcinogenesis. Although the nature of the constituents of the lipid fraction of WB that is responsible for the inhibition of colon carcinogenesis in the current study is not fully determined, one may speculate that the effects of this WB fraction may be attributable to one or several bioactive agents present in the bran oil. Additional studies are warranted to identify tumor-inhibitory component(s) in the lipid fraction of WB. The present study also demonstrates that WB-PF fortified with phytate has a minimal effect on colon tumor inhibition. Previous studies have demonstrated a protective effect of phytate against colon carcinogenesis (20, 21). However, in earlier studies, phytate at 1–2% levels was administered in drinking water to rats fed a low-fat, low-fiber diet (20, 21). The reasons for these divergent results could be attributable to differences in the route of administration of phytate (in drinking water versus diet), different basal diets (low-fat, low-fiber diet versus high-fat, WB-PF diet) and different amounts of phytate \((1–2% \text{ versus } 0.4\%)\).

The present study also demonstrated that the lipid fraction of WB-PF fortified with WB oil significantly suppresses both activities and expression of iNOS and COX-2 in colonic tumors. COX and iNOS play a very important role in colon tumor growth and progression (24, 25). In this connection, it is noteworthy that several human epidemiological studies have demonstrated that intake of NSAIDs, and among them especially aspirin, reduces the risk of colon cancer (31). Laboratory animal assays have supported this concept in studies with several NSAIDs (32). One of the mechanisms by which NSAIDs inhibit colon carcinogenesis is via inhibition of COX enzymes, which in a rate-limiting step catalyzes the conversion of AA into prostaglandins. The latter are potent biological mediators of diverse normal physiological effects and are implicated in various pathological conditions including inflammation and neoplastic transformation (33, 34). In addition, the byproducts of prostaglandin biosynthesis might be relevant mutagens (33). Several studies also indicate that nitric oxide enhances COX activity to produce proinflammatory prostaglandins that may lead to an exacerbated inflammatory response (35). A recent study by Amba et al. (25) suggests that excessive nitric oxide production by iNOS contributes to the pathogenesis of colon cancer progression at the transition of colon adenoma to carcinoma. Modulation of these enzyme activities by WB or its fractions has not been described in the literature. The mechanism of inhibition of iNOS and COX-2 by the lipid fraction of WB is not exactly known. Notably, the human diet intervention studies have demonstrated that certain dietary fibers such as WB affect the metabolic activity of the colonic microflora, mainly those that are involved in the production of putative colon tumor promoters. This effect depends on the type of fiber consumed (13, 14). It is likely that the suppression of colonic luminal pathogenic microbial activity by the lipid fraction leads to lesser inflammatory effects on the colonic epithelium. Human diet intervention studies also indicate that dietary WB but not oat bran or corn bran decreases the concentrations of fecal (colonic luminal) secondary bile acids, such as deoxycholic acid and lithocholic acid. These bile acids are potent promoters of colon carcinogenesis and stimulate the proliferation of colonic epithelium (13). These secondary bile acids have also been shown to increase the production of prostaglandin \(E_2\) and COX-2 expression. Secondary bile acid-mediated induction of COX-2 may to some extent explain the tumor-promoting effects of bile acids (36). The present study suggests that some of the bioactive agents present in the lipid fraction of WB possess anti-inflammatory properties and that the modulation of colon tumorigenesis by this fraction is associated with the inhibition of COX-2 and iNOS activities through its anti-inflammatory properties.

In conclusion, the study described here demonstrates for the first time that dietary administration of the lipid fraction of WB significantly inhibits AOM-induced colon tumor incidence, multiplicity, and volume, suggesting that this fraction contains compounds with putative chemopreventive properties against colon cancer. Although the exact mechanisms by which the lipid fraction of WB inhibits colon carcinogenesis remains to be elucidated, it would appear that the modulation of tumorigenesis by this fraction is associated with the alteration of iNOS and COX activities, thereby suppressing tumorigenesis. Thus, dietary inhibition of iNOS and COX-2 functions may provide protection against colon carcinogenesis. It is important for colon cancer prevention to fully identify all active components of WB oil that are responsible for colon tumor-inhibitory properties.

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