Effects of resistant starch and nonstarch polysaccharides on colonic luminal environment and genotoxin-induced apoptosis in the rat

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Fermentation of polysaccharides in the colon seems likely to regulate tumorigenesis but the mechanisms are unclear. A possible mechanism may be through facilitation of the acute apoptotic response to genotoxin-induced DNA damage. This study evaluated the effects of selected dietary polysaccharides, resistant starch (as Hi-maize) and nonstarch polysaccharides (as wheat bran and cellulose) on certain biological events relevant to protection against colon cancer (fecal bulk, pH, butyrate and apoptosis). Male Sprague–Dawley rats were fed the different experimental diets for a period of 4 weeks, after which a single azoxymethane injection was given to induce DNA damage; 6 h later the acute apoptotic response was measured. Other measures included short chain fatty acid (SCFA) levels, fecal bulk and pH. All wheat bran treatments significantly \((P < 0.05)\) enhanced carcinogen-induced apoptosis in the distal colon, increased fecal bulk and butyrate levels and reduced fecal pH, when compared with rats fed NF or Cellulose diets. Total SCFA \((P < 0.001, r = 0.496)\) and butyrate levels \((P < 0.001, r = 0.353)\) in the feces correlated positively with the acute apoptotic response in distal colonic crypts. Resistant starch supplementation by this modest amount did not enhance carcinogen-induced apoptosis. While it did significantly increase bulk, SCFA and butyrate levels and lower pH, the magnitude of these effects was not as great as with wheat bran. These findings indicate that wheat bran is the most effective regulator of these biological events of relevance to protection against colon cancer. Assuming that the acute apoptotic response to genotoxic carcinogens acts to remove genetically damaged cells that might otherwise form mutated clones that progress to malignancy, we have identified an additional biological mechanism by which dietary polysaccharides provide protection.

Introduction

Colorectal cancer has been suggested to be influenced strongly by dietary factors (1). Data from epidemiological studies (2–4) and experimental studies (5–7) have implicated dietary fiber as being protective against large bowel carcinogenesis although interventional studies in humans using non-starch polysaccharides (NSP) have showed varying results (8–11). The insoluble NSP sources such as wheat bran, which are slowly fermentable, tend to be more protective than the soluble (readily fermented) NSP sources such as oat bran, guar gum and pectin in the rodent models (6,12,13). The protective effects of the slowly fermentable NSP may relate to increased fecal bulk or reduced transit time (1,14), thereby diluting potential toxins and carcinogens and reducing their contact time with the colonic epithelium. Increased SCFA production, in particular butyrate, is another proposed mechanism for the protective effect of insoluble NSP (6). Butyrate, which is produced by anaerobic fermentation of NSP and other substrates in the colonic lumen has been shown to inhibit cell proliferation, induce differentiation and enhance apoptosis in colorectal cancer cells \(in vitro\) (15–17). Apoptosis appears to be a better predictor of tumor outcome than proliferation in induced carcinogenesis models (18). Reduced apoptotic ability to delete or remove cells with DNA damage (such as that due to carcinogen insult) may predispose to an increased risk of colorectal cancer (19), because mutated clones survive and may further progress to cancer. Environmental regulation of butyrate may thus regulate events in tumorigenesis.

The dietary component resistant starch (RS) resists small intestinal digestion and like NSP, reaches the colon undigested and be fermented by the colonic microflora to produce butyrate (20,21). Studies have shown that RS may act like soluble NSP in rodents (5,22) and is fermented rapidly in the cecum and proximal colon with little impact on distal colonic SCFA concentrations in the lumen. Because most tumors arise in the distal regions of the colon (23,24), for RS to be effective, it may need to have a greater effect on distal luminal conditions. However, whether RS can protect against colorectal cancer like some NSP sources remains unclear.

The purpose of the present study was to further explore the effects of RS (high amylose cornstarch) compared with variably fermented NSPs (α-cellulose and wheat bran) on colonic luminal environment along the length of the colon. In addition, we explored the relationship between changes in luminal environment, namely SCFA concentration, fecal pH, fecal bulk and fermentation, and certain epithelial events putatively related to colorectal cancer risk: specifically epithelial proliferation and the acute apoptotic response to genotoxic carcinogens. In particular, we were interested to see if increased distal luminal butyrate facilitated apoptotic deletion of genetically damaged cells.

Materials and methods

Animals and diets

A total of 72 male Sprague–Dawley rats, 6 weeks old were obtained from the Flinders Medical Centre Animal Facility, Adelaide, South Australia. Animals were divided randomly into six experimental groups and housed three per plastic cage in an animal holding room under controlled conditions of 22 \(\pm\) 2°C (SD), 80 10% humidity, and 12 h light/dark cycle. Animals were given free access to water and weighed weekly throughout the study.

The diets were based on the AIN-76 standard for purified diets for rats and mice (25). Each group of animals was fed one of six diets (Table I). The first group ‘NF’ consumed a diet containing no added fiber or RS. The second group ‘NF + RS’ contained RS in the form of high amylose cornstarch (Hi-maize) and was supplied by Penfold Australia Limited, Lane Cove, New

Abbreviations: AI, apoptotic index; AOM, azoxymethane; LI, labelling index; NF, no fiber; NSP, non-starch polysaccharide; RS, resistant starch; SCFA, short chain fatty acid; WB, wheat bran.
Table I. Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>NF</th>
<th>NF + RS</th>
<th>Cellulose</th>
<th>Cellulose + RS</th>
<th>WB</th>
<th>WB + RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>17.13</td>
<td>17.13</td>
</tr>
<tr>
<td>Corn starch</td>
<td>47.15</td>
<td>35.9</td>
<td>42.15</td>
<td>30.9</td>
<td>38.55</td>
<td>27.3</td>
</tr>
<tr>
<td>RS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>11.25</td>
<td>–</td>
<td>11.25</td>
<td>–</td>
<td>11.25</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>–</td>
<td>–</td>
<td>5</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11.19</td>
<td>11.19</td>
<td>11.19</td>
</tr>
<tr>
<td>Corn oil</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>17.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.95</td>
<td>10.95</td>
<td>10.95</td>
<td>10.95</td>
<td>10.73</td>
<td>10.73</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mineral mix&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Hi-maize cornstarch contains 61.8% resistant starch (45).

<sup>b</sup>AIN-76 vitamin and mineral mixtures.

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South Wales, Australia) and has been shown to contain 61.8% RS (43). Hi-maize (11.25 g/kg diet) was added to the diet at the expense of an equal amount of cornstarch (equivalent to 6.95% RS in the diet). The third group ‘Cellulose’ was fed α-cellulose (Sigma Chemical Company, St Louis, MO) at a level of 50 g/kg diet (equivalent to 5% total dietary fiber). The fourth group ‘Cellulose + RS’ was fed α-cellulose and Hi-maize. The fifth group ‘WB’ was fed wheat bran (Sanitarium Health Food Company, Australia). Wheat bran (44.7% total dietary fiber) was added to the diet to give a total dietary fiber level of 5%. The sixth group ‘WB + RS’ was fed wheat bran and Hi-maize.

**Experimental procedure**

After 3 weeks on experimental diets rats were housed temporarily in metabolic cages and food intake and fecal output were measured for 48 h. On the last 3 days of the experimental period fresh fecal samples were collected from each rat by gently handling the rats until they produced a fecal sample. For fecal pH and SCFA analysis, the fresh feces were placed immediately in 2 ml of cold saline, homogenized, pH measured (TPB, digital pH meter, model 1852 mV, Brisbane, Australia) and stored at −20°C.

After 4 weeks on experimental diets each rat received a single i.p. injection of AOM (10 mg/kg body weight, Sigma Chemical Co., St Louis, MO). Rats were killed by CO2-induced narcosis, 6 h after AOM treatment. Immediately after death, the entire colon was rapidly removed and divided into proximal and distal portions; the limit of the proximal portion was defined by the ‘herring bone’ pattern. These were flushed clean with ice-cold saline. Segments of 2 cm were taken from the cecal end of the proximal portion and the rectal end of the distal portion. These segments were placed in 10% buffered formalin for 24 h, then washed and stored in 70% ethanol. The cecum was excised, weighed and a known weight of content placed in 2 ml of cold saline for pH measurement and samples stored at −20°C for SCFA analysis.

The Flinders University of South Australia Animals Welfare Committee approved all experimental procedures.

**Evaluation of apoptosis**

Colon sections (0.5 cm × 0.5 cm) in 70% ethanol were cut from proximal and distal segments of the colon and embedded in paraffin. Paraffin-embedded sections (5 μm) were stained with hematoxylin and evaluated under a light microscope for apoptotic cells. Apoptotic cells were identified in 20 randomly chosen intact crypts by cell shrinkage, presence of condensed chromatin and sharply delineated cell borders surrounded with a clear halo (Figure 1) as described by Potten et al. (26). The percentage of apoptotic nuclei (apoptotic index) was calculated as the mean number of apoptotic cells/crypt column multiplied by 100. The length of each crypt was determined along with the position of apoptotic cells.

**Determination of cell proliferation**

To assess the proliferative activity and the distribution of proliferating cells in the colonic crypts the proliferating cell nuclear antigen (PCNA) was performed using standard immunohistochemical procedures. Briefly, deparaffinized sections were rehydrated in a graded series of ethanol from 100% to 50% and then to distilled water. The primary mouse monoclonal antibody (PC-10, Santa Cruz, USA) was placed on the slides (1/500 dilution) and incubated overnight. Endogenous peroxidase activity was blocked by immersing the slides in 1% H2O2 in 50% ethanol for 10 min and then washed with distilled water. A Level 2 Ultra Streptavidin detection system (Signet Laboratories, USA) was used utilizing biotinylated goat anti-mouse as the secondary antibody. The slides were counterstained for 3 min with haematoxylin.

The scoring for cell proliferation was the same as the method used to score apoptosis.

**SCFA analysis**

Fecal and cecal samples were homogenized in 4 volumes of internal standard solution (heptanoic acid, 3.5 mM) and centrifuged at 3000×g for 10 min.
The supernatant was then distilled and 0.3 ml injected into a gas chromatograph (Hewlett Packard 5890 Series II A) equipped with a flame ionization detector and a capillary column (Zebron ZB-FFAP, 30 m × 0.53 mm ID, 1 μm film, SGE, Australia). Helium was used as the carrier gas, the initial oven temperature was 120°C and was increased at 20°C per minute to 180°C, the injector temperature was 200°C and the detector temperature was 200°C. A standard SCFA mixture containing acetate, propionate and butyrate was used for calculation and the results are expressed as µmol/g of sample.

### Statistical analysis

Data are expressed as mean ± SD, and differences between means were analysed by ANOVA, with statistical differences separated by least significant differences using SPSS version 10 software. When variances were not homogeneous by Bartlett test, the data were logarithmically transformed and transformed data were then analysed by ANOVA followed by multiple comparison. The correlations among variables were analysed by Spearman’s correlation test. Differences were considered significant at P < 0.05.

### Results

#### Bodyweight and food intake

All treatment diets were well tolerated and there were no significant differences among the different groups in the quantities of food eaten or bodyweight gained (Table II).

#### Fecal output and fecal pH

Addition of RS, Cellulose and WB to the NF diet significantly increased fecal output (P < 0.001) (Table II). In the three groups of RS fed rats, further increases in fecal output was seen with the addition of Cellulose (P = 0.005) or WB (P = 0.002). The largest incremental increase was with RS together with WB and this was greater than either RS or WB alone.

Fecal pH was used as an indicator of distal lumenal pH (35) and is shown in Table II. The addition of RS and WB but not Cellulose to the NF diet significantly decreased fecal pH (P = 0.001). The addition of Cellulose and WB to the RS fed rats did not further change pH compared with RS alone.

#### Cecal weight, content and pH

The addition of RS to the NF diet significantly increased cecal wall weight (P = 0.013), however no significant increase in cecal weight was observed with the addition of Cellulose or WB (Table II). The addition of Cellulose and WB to the RS diets significantly increased cecal wall weight in both treatment groups compared with the NF controls (P < 0.001) but not above that of RS without NSP.

The addition of RS and WB to the NF diet significantly increased cecal content weight (P < 0.01) while no difference was seen with the addition of Cellulose (Table II). The supplementation of Cellulose and WB to the RS diet significantly increased cecal content weight (P < 0.01), with the WB + RS group having the largest content, which was greater than either of the dietary components alone.

Cecal pH (Table II) was significantly decreased with the addition of RS and WB to the NF diet (P < 0.001) while no difference was seen with Cellulose addition. When RS fed rats were further supplemented with either Cellulose or WB, cecal pH levels were not different from RS alone.

#### Cecal SCFA

The results of SCFA analysis in the cecal content are expressed as µmol/g and are shown in Table III. The addition of RS, WB or Cellulose alone to the NF diet failed to significantly alter the total cecal SCFA concentration in the cecum. When Cellulose or WB were combined with the RS diet, a significant increase in total SCFA was observed compared with the NF control (P < 0.01) only in the WB + RS group of rats.

Of the individual SCFA, butyrate was the most altered by the diet. The addition of RS and WB to the NF diet significantly increased cecal butyrate concentration (P < 0.01), no difference was seen with the addition of Cellulose (Table III). The supplementation of the RS diets with Cellulose or WB resulted in a further increase in cecal butyrate only in the WB + RS group of rats (P < 0.01). Cecal acetate and propionate levels were less affected by the diet. Cellulose consistently caused the lowest levels of all SCFA, significantly less than RS for all three SCFA.

#### Fecal SCFA

The results of SCFA analysis in the feces are shown in Table III. The addition of WB to the NF diet resulted in a significant increase in total fecal SCFA concentration (P < 0.01), although there was no significant difference when compared with the NF + RS treatment. No significant increases were observed when RS or Cellulose were added to the NF diet. Supplementation of the RS diet with WB or Cellulose resulted in a further increase in total SCFA concentration in the RS + WB group of rats (P < 0.01), although this was not significantly different from the group of rats fed WB alone or WB + RS.

Fecal butyrate concentration was shown to significantly increase when WB was added to the NF diet (P < 0.01), however there was no significant difference in butyrate concentration between the WB treatment and the NF + RS treatment group. No significant changes were seen when RS or Cellulose was added to the NF diet (Table III). Supplementation to the RS diet with WB resulted in further increases in fecal butyrate (P < 0.001). The highest concentration was observed in the

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**Table II. Effects of no fiber (NF), Cellulose, wheat bran (WB) and resistant starch (RS) on food intake, body weight gain, fecal output, fecal pH, cecal weight, cecal content and cecal pH in rats**

<table>
<thead>
<tr>
<th>Diet</th>
<th>NF</th>
<th>NF + RS</th>
<th>Cellulose</th>
<th>Cellulose + RS</th>
<th>WB</th>
<th>WB + RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/day)</td>
<td>19.9 ± 1.5</td>
<td>19.0 ± 1.0</td>
<td>21.8 ± 2.4</td>
<td>21.2 ± 1.1</td>
<td>20.6 ± 1.3</td>
<td>21.6 ± 1.4</td>
</tr>
<tr>
<td>Weight gain (g/rat/wk)</td>
<td>39.5 ± 4.1</td>
<td>34.1 ± 3.5</td>
<td>36.5 ± 5.8</td>
<td>36.3 ± 4.5</td>
<td>40.8 ± 5.4</td>
<td>40.4 ± 4.3</td>
</tr>
<tr>
<td>Fecal output (g/day)</td>
<td>0.3 ± 0.2a</td>
<td>1.2 ± 0.6b</td>
<td>1.7 ± 0.3b</td>
<td>2.6 ± 0.5c</td>
<td>2.5 ± 0.5c</td>
<td>3.6 ± 0.6d</td>
</tr>
<tr>
<td>Fecal pH</td>
<td>7.3 ± 0.1a</td>
<td>6.7 ± 0.2a</td>
<td>7.2 ± 0.1a</td>
<td>6.6 ± 0.2a</td>
<td>6.9 ± 0.1b</td>
<td>6.6 ± 0.2a</td>
</tr>
<tr>
<td>Cecum weight (g)</td>
<td>0.55 ± 0.1a</td>
<td>0.63 ± 0.1b</td>
<td>0.53 ± 0.1a</td>
<td>0.64 ± 0.1b</td>
<td>0.56 ± 0.1a</td>
<td>0.67 ± 0.1b</td>
</tr>
<tr>
<td>Cecal contents (g)</td>
<td>1.7 ± 0.5a</td>
<td>2.2 ± 0.4a</td>
<td>1.7 ± 0.4a</td>
<td>2.4 ± 0.6a</td>
<td>2.4 ± 0.4b</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>Cecal pH</td>
<td>7.2 ± 0.2a</td>
<td>6.6 ± 0.5d</td>
<td>7.1 ± 0.2d</td>
<td>6.8 ± 0.2b</td>
<td>6.9 ± 0.1b</td>
<td>6.8 ± 0.2b</td>
</tr>
</tbody>
</table>

aData are expressed as means ± SD (n = 12); values in a row without a common superscript letter are significantly different (P < 0.05).
Acetate 12.5/H11006/H11006
Propionate 9.1 ± 3.1/H11006/H11006
Butyrate 4.4 ± 1.6/H11006
Total SCFA 42.5 ± 16/H11006/H11006

Data are expressed as means ± SD (n = 12); values in a row without a common superscript letter are significantly different (P < 0.05).

WB + RS group with a doubling of fecal butyrate, compared with those rats fed WB alone.

**Apoptosis and cell proliferation**

A significant increase in the apoptotic index (AI) in the distal colon (Figure 2) was observed in the WB fed rats (P = 0.015). The addition of RS to the NF, Cellulose and WB treatments had no further effects on apoptosis in the distal colon.

No significant difference in the AI was observed in the proximal colon between the different individual dietary treatment groups (Figure 3). Significantly higher (P < 0.001) AI was observed in the distal colon when compared with the proximal region of the colon.

As shown in Figure 2, PCNA labelling index and crypt height in the distal colon did not differ among the different dietary treatment groups. Crypt height did not differ in the proximal colon; PCNA labelling index was not measured in this segment, as there was no difference in the AI between the different treatments.

**Association of cecal and fecal parameters with apoptosis in the distal colon**

The AI of the distal colon was found to be significantly associated with a number of parameters in the cecum and feces after controlling for the effect of the different diets (Table IV). The strongest relationships were seen between fecal SCFA (total and individual) and distal AI. Relationships between cecal lumenal events and AI in the distal colon were weaker, and were seen only for butyrate.

**Discussion**

The present study has demonstrated that feeding wheat bran can facilitate the acute apoptotic response to genotoxic carcinogens in the distal colon of rats and this appears to be dependent on fermentation. Apoptosis is an important biological regulatory process in the protection against the development of cancer and has been suggested to be a better predictor of tumor outcome than cell proliferation in carcinogen-induced models (18). Upregulation of apoptosis during the initiating step of colorectal cancer may result in increased elimination of the DNA-damaged cells that might otherwise progress to malignancy.

Previous animal studies have shown that dietary wheat bran can protect against the development of colorectal cancer (6,12,27,28). Our data suggest that the acute apoptotic response to genotoxin-induced damage can be upregulated by wheat bran, thereby providing a possible biological explanation for how wheat bran protects, i.e. it facilitates deletion of mutated cells.

Other studies (27,29) have recently shown an increase in certain types of apoptosis with feeding of wheat bran. However, it must be pointed out that these studies have measured apoptosis in the rat colon at other different stages of tumorigenesis than in the present study, in particular, they have not examined the acute apoptotic response to genotoxic carcinogen. Compner et al. (27) showed that wheat bran protected against the development of precursor lesions (aberrant crypt foci) of colon cancer and that apoptosis in non-tumor colonic epithelium was increased 5 days after injection of carcinogen. Jenab and Thompson (29) showed that wheat bran increased apoptosis at 100 days post-initiation. Our previous studies (Hu et al., submitted for publication) and that of Hirose et al. (30) have shown that spontaneous apoptosis in the distal colon epithelium of animals not receiving AOM is very low (i.e. below 0.5%). Hu et al. also found that dietary treatment did not alter this form of apoptosis. The peak time for the acute apoptotic response to carcinogen was found to be 6–8 h post AOM injection and that by 72 h the level of apoptosis is returning to normal (Hu et al., submitted for publication, 30). These other studies (27,29) have thus measured a different form of apoptosis that does not directly reflect the DNA damage caused by the initiating carcinogen, certainly such apoptosis is not in direct response to initiation-related events. Ishizuka et al. (27), submitted for publication, 30). These other studies (27,29) have thus measured a different form of apoptosis that does not directly reflect the DNA damage caused by the initiating carcinogen, certainly such apoptosis is not in direct response to initiation-related events.

In the present study the acute apoptotic response to the genotoxic carcinogen did not change relative to fiber-free and RS-free diet, when rats were fed a diet supplemented with α-cellulose or RS. This suggests that in the amounts used, neither the non-starch polysaccharide α-cellulose nor RS from Hi-maize™ are offering protection against colorectal cancer.
through regulation of acute apoptotic response to the genotoxic carcinogen. It must be emphasized that though we used only a modest concentration of RS in this study, higher concentrations of RS may be required to reproduce the type of fermentation seen with wheat bran. Wheat bran in contrast to RS and \(\alpha\)-cellulose, was effective in facilitating apoptosis in the distal colon and our results give some clues as to the possible mechanism. Wheat bran feeding significantly increased the total SCFA levels including butyrate in the feces of rats when compared with rats fed a no-fiber diet. Fecal SCFA levels correlate with distal colonic lumenal levels (35). When regression analysis was performed regardless of the dietary group, total SCFA and butyrate levels in the feces were correlated with carcinogen-induced apoptosis in the distal colon. This suggests that a fermentation-related event is

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**Table IV. Correlations between apoptotic index (AI) in the distal colon and selected fecal and cecal parameters**

| Apoptotic index (%) | \(r\) | \(r\)  
|---------------------|-----|-----
| Feces              |     |     
| Total SCFA (\(\mu\)mol/g) | 0.50** |     
| Acetate            | 0.43** |     
| Propionate         | 0.56** |     
| Butyrate           | 0.41** |     
| Cecum              |     |     
| Total SCFA         | NS |     
| Acetate            | NS |     
| Propionate         | NS |     
| Butyrate           | 0.24* |     

*Analysis was done by partial correlation controlling for different diets. NS = no significant correlation \((P > 0.05)\). *\(P < 0.05\), **\(P < 0.001\). SCFA, short-chain fatty acids.
responsible for the increase in apoptosis, especially considering that α-cellulose did not undergo fermentation and did not increase apoptosis. Other studies have also shown that wheat bran increases butyrate levels (27,35). Butyrate has been shown to induce differentiation and suppress proliferation in colon cancer cells in vitro (17,36); acetate and propionate are much less potent. There is also evidence that butyrate induction of apoptosis in colon cancer cells is probably independent of p53 (15,37). Butyrate achieves this by inhibition of histone deacetylase (15). Such studies strongly suggest that butyrate is the specific facilitator of this form of apoptosis. It is likely that the apoptosis observed in the present study following carcinogen exposure is p53-dependent. Studies in mice following a genotoxic event in the form of ionizing radiation exposure demonstrated that p53 protein is expressed in the crypts of both the colon and small intestine and this p53 expression was shown to correlate with apoptosis (38). The authors also showed that in mice without copies of the p53 gene, apoptosis was completely abrogated following radiation exposure.

Fermentation outcomes may not be the only mechanism by which wheat bran protects against colorectal cancer (39). Components associated with fiber such as phytochemicals may be also responsible for the protective effect of wheat bran (29,40). They also have the potential to regulate events at the time of initiation by carcinogen (41).

Our data did not show any alteration in distal epithelial proliferation. This result demonstrates that the facilitation of apoptosis by wheat bran is occurring independent of cell proliferation. Expansion of mucosal cell proliferation has been suggested as a risk factor for colon cancer (42). However other studies have raised doubt whether cell proliferation is a reliable predictor of cancer risk (18,43) and that apoptosis may be a more reliable measure of risk (18). Certainly, the increase in apoptosis is not an artifact of increased proliferation exposing DNA to more damage by the carcinogen.

Although RS at a level of 6.95% in the diet did not alter the acute apoptotic response to the genotoxic carcinogen in this study it did significantly affect other measures that are considered beneficial in terms of bowel health. Supplementation of the no-fiber diet with only a modest amount of RS significantly increased fecal bulk and lowered fecal pH. Both factors have been speculated to be mechanisms by which NSP exert their protective effect on colon cancer (14,44). Fermentation of RS was greater in the cecum compared with the feces, and this was evident by lower pH, higher caecal content and caecal SCFA levels. Ferguson and coworkers (45) also reported significant changes in fecal pH and bulking as well as greater production of SCFA in the cecum of rats fed RS preparations. They suggested that RS produces effects on gastrointestinal tract function in the rat model that more closely resemble the effects of soluble dietary fiber than that of the insoluble dietary fiber, wheat bran. However, we found that when RS was combined with WB, much higher SCFA levels, in particular butyrate was observed in the feces. Other researchers (22,46) have also observed this phenomenon. Morita et al. (46) reported in rats that when RS was combined with psyllium, the site of RS fermentation was pushed more distally. Govers et al. (22) found that WB could shift the fermentation of RS further distally in pigs. As the distal colon is the site where most tumors arise (23,24) it may be of additional benefit for cancer protection if fermentation is further enhanced within the distal colon. Clearly, further studies are needed to examine this hypothesis and especially to determine if large amounts of RS or different types of RS are able to mimic the effect of wheat bran in the distal colon.

In summary, our results show that wheat bran facilitates the acute apoptotic response to a genotoxic carcinogen in the distal colon. A possible mechanism for this effect is increased fermentative production of SCFA, particularly butyrate. Wheat bran also pushes the fermentation of RS into the distal colon, which may be of additional benefit to colorectal cancer protection. Further studies are necessary to determine if genotoxin-induced apoptosis can be linked to the production of butyrate in a concentration-dependent manner, whether other dietary factors that raise butyrate also facilitate apoptosis, and whether dietary regulation of apoptosis at the time of initiation is a significant determinant of subsequent events.

Acknowledgement

This investigation was supported by Penford Australia Limited.

References


Received September 6, 2001; revised December 28, 2001; accepted January 18, 2002