Reports

Enhancement of 1,2-Dimethylhydrazine-Induced Large Bowel Tumorigenesis in Balb/c Mice by Corn, Soybean, and Wheat Brans

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Abstract

This study was designed to determine the effects of four well-characterized dietary brans on large bowel tumorigenesis induced in mice with 1,2-dimethylhydrazine (DMH). Eight-week-old barrier-derived male Balb/c mice were fed a semisynthetic diet with 20% bran added (either corn, soybean, soft winter wheat, or hard spring wheat) or a no-fiber-added control diet. Half of each group was given DMH (20 mg/kg body weight/week, subcutaneously for 10 weeks) beginning at 11 weeks of age. Surviving mice were killed 40 weeks after the first DMH injection. Tumors were not found in mice not subjected to DMH. In DMH-treated mice, tumors were found almost exclusively in the distal colon. Tumor incidences were as follows: controls, 11%; soybean group, 44%; soft winter wheat group, 48%; hard spring wheat group, 58%; and corn group, 72%. Tumors per tumor-bearing mouse ranged from 1.4 to 1.6, except in the corn group, which had 2.1. A positive correlation was found between percentage of neutral detergent fiber in the brans and tumor incidences but not between the individual components of cellulose, hemicellulose, or lignin. The enhancement of DMH-induced large bowel tumorigenesis by all four bran types may reflect a species and/or mouse strain effect that is bran-source related. These data emphasize the importance of using well-defined bran in all "fiber" studies.

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Introduction

Among the various forms of malignancy, cancer of the colon ranks second only to lung cancer in men and breast cancer in women as a cause of death. It accounts for 15% of all cancers and is projected to be the cause of death in about 57,000 people in the United States annually[1].

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Epidemiologic studies[2,3] suggest that genetic factors play only a minor role in the causation of most human colon cancer, with the exception of familial polyposis coli. On the other hand, dietary factors—in particular, high dietary fat[4–7] and protein[6,8] and lack of dietary fiber[9–11] are thought to play a major role in colon carcinogenesis. To date, there has been no consistent negative correlation of tumor incidence with high-fiber diets experimentally[12]. Further, there has been little definitive study with different kinds of bran to show which might be more effective in modifying colon carcinogenesis[13].

Conflicting information has been reported regarding the effect of dietary fiber on colon tumorigenesis in animal models. Some studies demonstrated that wheat bran[14–21] and cellulose[15,22,23] decreased chemically induced large bowel tumor yield, whereas others found no protective effect[19,24–26]. In some studies, decreases in incidences of benign colon adenomas by wheat bran have been observed[19–21], in contrast with other studies demonstrating a failure of wheat bran to reduce colonic adenocarcinomas[14,20,21,25,26]. Further, some studies reported increased tumor yield with an increase in dietary fiber[13,19,27]; in other studies, fiber-free diets were even shown to protect against colon cancer[16,28]. The above findings suggest that biological activity depends upon fiber source, amount of fiber given, type of carcinogen used, and species of animal treated. Thus, the dietary factors in colon carcinogenesis are anything but unequivocal, and they certainly appear complex in their interactions.

A recent study using different well-characterized bran sources in rats[13] showed protection only when wheat bran was added after DMH treatment was completed (promotion phase of carcinogenesis). None of the four bran types used (wheat, soybean, rice, or corn) protected when started prior to DMH treatment and given for life. The study used a high-fat diet (20% corn oil) similar in percentage to Western human diets and a treatment regimen of two relatively high doses of DMH. Despite similar tumor incidences in the control and treatment groups (incidences increased from 93% in controls to 100% in the group of DMH-treated rats given corn bran), the significantly increased number of tumors per tumor-bearing rat suggested an enhancing effect of corn bran on DMH tumorigenesis. The reason for this is unclear.

Discussion of the above reports indicates that definitive data are still needed to elucidate the effect of interactions between varied fiber levels and other nutrients (such as fat), as well as the effect of fiber source, on colon cancer induction. The need to use well-characterized fiber sources becomes obvious if responsible fiber components are to be identified as either beneficial or detrimental. Further, the use of another species (e.g., mouse) would complement data and conclusions from studies using rats, as well as correlations with observations in humans and ultimate application to the human disease.

Thus, the current study was designed to determine the effects of four well-characterized dietary brans on large bowel tumorigenesis induced by DMH in mice. In contrast to previous studies, the protocol used in the present study included a lower dietary fat (5%) and a lower total dose of DMH (200 mg/kg body weight in mice vs. 300 mg/kg body weight in rats) given over a 10-week period. These treatment regimens were used to provide favorable conditions for demonstrating any existing protective effect of the brans.

Materials and Methods

Four-week-old barrier-derived male Balb/c mice (Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN) were acclimated for 4 weeks and then placed on either a semisynthetic diet (US Biochemical Corp., Cleveland, OH) to which one of four brans was added—corn, soybean, soft winter wheat, or hard spring wheat—or a no-fiber-added control diet.

Diets (Table 1) were modifications of the AIN-76 recommended mouse diets[29] with low fat (5% beef tallow); bran was added in lieu of carbohydrate sources, cornstarch and dextrose. Based upon estimated values of 4 calories/g for carbohydrates and protein and 9

Table 1. Percentage Composition of Semisynthetic Diets

Component	Diet				
	Control	Spring wheat bran	Winter wheat bran	Corn bran	Soybean bran
Casein (vitamin-free)	22.0	22.0	22.0	22.0	22.0
DL-methionine	0.3	0.3	0.3	0.3	0.3
Cornstarch	51.0	36.0	36.0	36.0	36.0
Dextrose	17.0	12.0	12.0	12.0	12.0
Beef tallow	5.0	5.0	5.0	5.0	5.0
Mineral mixture[a]	3.5	3.5	3.5	3.5	3.5
Vitamin mixture[a]	1.0	1.0	1.0	1.0	1.0
Choline Bitartrate	0.2	0.2	0.2	0.2	0.2
Spring wheat bran	0.0	20.0	0.0	0.0	0.0
Winter wheat bran	0.0	0.0	20.0	0.0	0.0
Corn bran	0.0	0.0	0.0	20.0	0.0
Soybean bran	0.0	0.0	0.0	0.0	20.0

a: Vitamin and mineral mixtures were formulated according to American Institute of Nutrition recommendations [29].

calories/g for fat, there were 4.0 calories/g in the control diet and 3.6 calories/g in the bran diets.

Soft winter and hard spring wheat brans, which were used because of observed differences in cholesterol metabolism studies[30], were provided by the American Association of Cereal Chemists (St. Paul, MN). Feed-grade soybean hulls and animal-grade unrefined corn bran were supplied by Staley Manufacturing Company (Decatur, IL). Brans were ground using a US No. 30 screen to decrease the variation in particle size, which is known to influence fecal bulking and hydration and intestinal transit time[31,32]. Diets were stored at 5°C until a few days before use.

Chemical analysis of the four brans used in the study is shown in Table 2. Considerable variations were apparent between brans from different plant sources. Fat content was one component that was relatively constant in all brans.

Beginning at 11 weeks of age, half the animals in each diet group were given 1,2-dimethylhydrazine (DMH) dihydrochloride (20 mg/kg body weight/week; Aldrich Chemical Company, Milwaukee, WI) for 10 successive weeks. The DMH was administered subcutaneously in physiological saline solution. The other half of each group received injections of saline alone.

Surviving animals were killed at 51 weeks of age (40 weeks after the first DMH injection). Colons were inflated in situ with Telly's fixative, and then cleared and stained with Wright's stain as previously described[33]. Tumors were identified under a dissecting microscope and located on a schematic drawing of the colon. Sections of the tumor-bearing segments and of the nontumorous colons were processed and stained with hematoxylin and eosin and special stains, when indicated, such as periodic acid Schiff. Tumors were then evaluated histologically as adenomas or adenocarcinomas.

Results

Weight gains in all groups were identical, as seen in Figure 1, and were typical of male Balb/c mice. No differences existed between groups given added bran in the diet or DMH or any combination thereof. There was no suggestion of any dietary deficiencies.

Table 2. Chemical Analysis of Corn, Soybean, Soft Winter Wheat, and Hard Spring Wheat Brans

Component	Bran source			
	Corn bran[a] (%)	Soybean bran[a] (%)	Soft winter wheat[b] (%)	Hard spring wheat[b] (%)
Crude fiber	14.81	44.28	10.20	10.30
Ash	1.06	4.94	6.00	7.02
Protein	7.05	9.20	15.01	16.61
Fat	1.32	1.24	1.20	1.30
Oil	0.95	0.70	—	—
Starch	16.25	—	20.00	16.00
Neutral detergent fiber	78.80	66.56	38.30	43.20
Acid detergent fiber	19.01	54.00	11.10	13.70
Acid detergent lignin	0.86	2.32	2.80	4.10

a: Supplied and analyzed by Dr. R.R. Hahn, A.E. Staley Manufacturing Company, Decatur, IL. Analysis performed on dry substance basis.
b: Analyzed by Medallion Laboratories for the American Association of Cereal Chemists. Analysis performed on "as is" basis (water not removed prior to analysis).

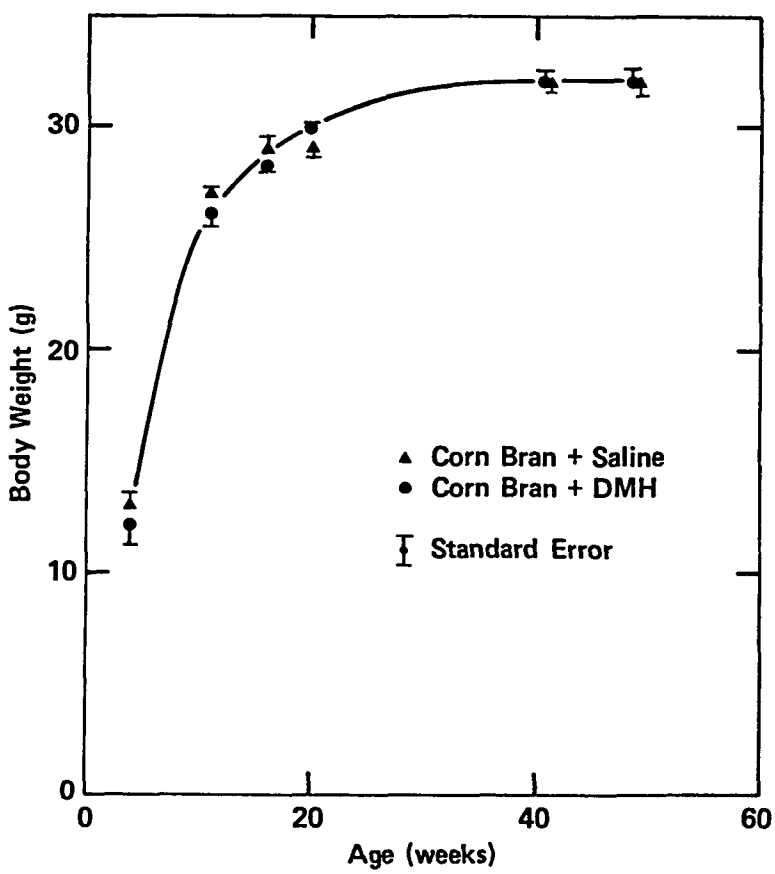

Figure 1. Weights of male Balb/c mice given corn bran ± DMH. (Comparative weights for no-bran-added diet ± DMH were 29 ± 0.7 g and 30 ± 0.5 g at 20 weeks, 31 ± 0.8 g and 32 ± 0.15 g at 49 weeks.

With the exception of the hard spring wheat group, survival was generally lower in DMH animals regardless of the bran type, but no survival rates were statistically different from controls (Table 3). Colon tumors were not found in mice not subjected to DMH treatment. In tumor-bearing mice, 12% of the tumors (a range of 6%–21% between diet groups) were adenocarcinomas, and 88% were adenomas. (A diagnosis of adenocarcinoma was made when foci were very anaplastic, cells were disoriented, and mitotic activity was markedly increased.) Whereas all larger tumors (~5 mm) were polypoid, smaller tumors (≤ 1 mm) were often sessile and had more normal cellular orientation. Adenocarcinomas often had distinct foci of mucinous and nonmucinous tumor cells within a single tumor. Metastases were relatively rare as was local invasion into the muscularis mucosa.

Table 3. Colon Tumors Induced by DMH in Balb/c Mice as Affected by 20% Added Bran in the Diets

Treatment group		Percentage of mice surviving to sacrifice	Colon tumors		
DMH	Bran		Percentage of mice with tumors	Total no. tumors	Tumors/ tumor-bearing mouse
+	0	82 (47/57)	11 (5/47)	7	1.4
0	0	95 (55/58)	0 (0/55)	0	0
+	Soybean	81 (57/70)	44 (25/57)	40	1.6
0	Soybean	90 (64/71)	0 (0/64)	0	0
+	Soft winter wheat	77 (50/65)	48 (24/50)	39	1.6
0	Soft winter wheat	88 (60/68)	0 (0/60)	0	0
+	Corn	80 (39/49)	72 (28/39)	60	2.1
0	Corn	90 (44/49)	0 (0/44)	0	0
+	Hard spring wheat	86 (43/50)	58 (25/43)	34	1.4
0	Hard spring wheat	80 (40/50)	0 (0/40)	0	0

The tumor incidence in the DMH-treated control-diet group was 11%; other DMH-treated groups showed increasing tumor incidences, as follows: soybean (44%), soft winter wheat (48%), hard spring wheat (58%), and corn (72%). The number of tumors/tumor-bearing animal ranged from 1.4 to 1.6 for all treated groups except the corn bran group, which had 2.1. DMH-treated animals dying before scheduled sacrifice had few tumors (3/13) if death occurred before 40 weeks of age (Table 4), but 18 of the 39 who died from 40 to 51 weeks of age had tumors. The number of tumors/tumor-bearing mouse was 2.0 in the soft winter wheat group and 3.5 in the corn bran group.

The percentage of neutral detergent fiber was the only factor in the composition of the brans that correlated with tumor incidences (Figure 2).

Table 4. Colon Tumors in Mice Dying Prior to Sacrifice Time

Treatment group		Mortality data						
		Mice dying at 20–39 weeks			Mice dying at 40–51 weeks			
DMH	Bran	No.	No. with tumors	Total no. tumors/ group	No.	No. with tumors	Total no. tumors/ group	Tumors/ tumor-bearing mouse
+	0	1	1	1	10	5	8	1.6
0	0	8	—	—	0	—	—	—
+	Soybean	3	1	1	10	4	4	1.0
0	Soybean	4	—	—	3	—	—	—
+	Corn	3	—	—	5	4	13	3.25
0	Corn	1	—	—	4	—	—	—
+	Hard spring wheat	0	—	—	6	3	4	1.3
0	Hard spring wheat	3	—	—	7	—	—	—
+	Soft winter wheat	6	1	3	8	2	4	2.0
0	Soft winter wheat	5	—	—	4	—	—	—

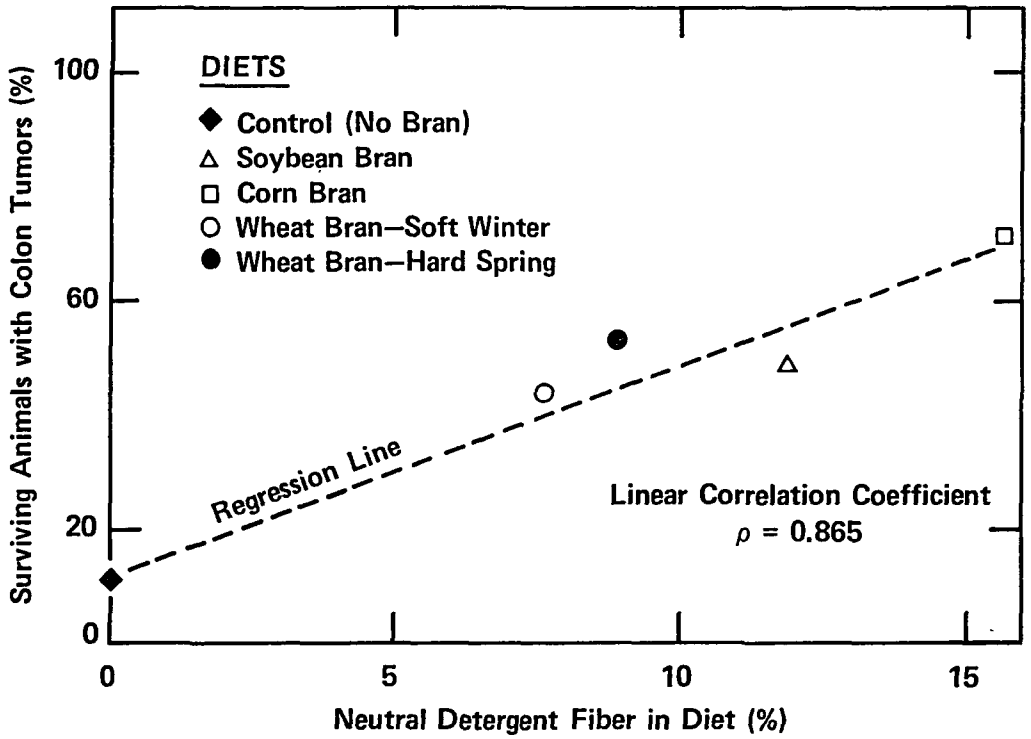

Figure 2. Relationship between neutral detergent fiber in bran diets and tumor incidences in mice receiving the diets.

Discussion

The fact that the growth curves for all groups were identical (Figure 1) suggests that no detectable nutritional deficiencies existed and that no modification of tumor yield occurred because of forced caloric reduction due to added bran. Admittedly, weight gain is a crude measure, but the absence of altered growth curves was strongly suggestive that mice received adequate nutrient (and especially caloric) requirements.

Colon tumor incidences were somewhat surprising (Table 3). The group of DMH mice on the control diet had an 11% incidence, which was below our previous observations[33]. In our earlier study, we fed Balb/c mice Purina rodent laboratory chow 5001 with and without added butylated hydroxytoluene (BHT) and treated the mice with DMH. The exact percentage of fat in chow 5001 was unknown, but was listed as "crude fat not less than 4.5%." In the current study, mice from all five diet groups were given DMH simultaneously. Within each bran diet group, each of the 6–7 cages of mice had nearly identical tumor incidences. Thus, the differences in tumor incidences between the two experiments may be related to dietary differences, e.g., the amount of dietary fat, which, in the present study, was rigidly controlled at 5% added beef tallow, a low risk (or "protective") level; the absolute amount in the previous study was not known. Another dietary variable was that the Purina chow control diet[33] had $\sim$ 5% crude fiber whereas the semisynthetic control diet in the current study had 0% fiber. Potential dietary interactions between fat and fiber in colon carcinogenesis have been suggested by Nigro et al.[15] and may, along with other unidentified factors, have contributed to these differences.

The enhancement of colon tumor incidences in all bran groups was quite unexpected. Although the methods of the present study differed somewhat from those used by Barnes et al.[13], their research (using rats) showed no consistent protective effect for bran. They

observed bran protection only when wheat bran was given after DMH treatment. Similar to the observations reported herein in mice, corn bran increased tumor yield in rats. The greater tumor-enhancing effect of corn bran was not as clear, however, in the study by Barnes et al.[13] as in our study, because tumor incidences in the rats were very high—75%–93%. Had a less effective treatment protocol been used, a more easily recognized enhancement might have been seen in the rats, such as we observed in mice. Conceivably, high doses of DMH and high fat levels could overwhelm any protective effect, as suggested by Nigro et al.[15].

In spite of low fat and high fiber diets in the current study, all of the bran diets actually enhanced tumor yield, with corn bran having the greatest effect. Few fiber studies have been done in mice; consequently, these results may reflect a species and/or mouse strain effect. However, a sex-dependent BHT protective effect in DMH-induced colon carcinogenesis was observed in Balb/c males in a previous study[33]. Also, Chen reported a protective effect in CF_1 female mice from a 40% wheat bran diet of undetermined source, but the DMH treatment regimen was very toxic—$\sim$65% mortality in $<$15 weeks—with a treatment period of 26 weeks[17]. Jacobs' recent report suggesting hyperproliferative effects of wheat bran when given during DMH treatment could account, in part, for the increase in tumor incidences by all four brans used in the current study as well as the increases he observed[34].

Neutral detergent fiber includes principally cellulose, water-insoluble hemicellulose and lignin whereas acid detergent fiber includes only cellulose and lignin. No correlations occurred between tumor incidences and hemicellulose, cellulose, or lignin. Only the percentage of neutral detergent fiber (the components in combination) correlated with tumor incidences. The significance of this observation is uncertain at this time but does show the need for using well-defined bran in all fiber studies. The complexities of bran (or fiber) and the physical and physiological differences between types are evident from this and other studies, suggesting that physiologic activity may be affected not only by the size of the bran particles but also by the source of the bran and the amounts of each component.

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