Note

Inhibitory Effect of Dietary Wheat Bran on Formation of Aberrant Crypt Foci in Rat Colon Induced by a Single Injection of 1,2-Dimethylhydrazine

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The frequency of appearance of aberrant crypt foci (ACF) in the distal colon was significantly lower in rats fed a high fiber (20% wheat bran) diet than in those fed a fiber-free one at 4 weeks after a single injection of 1,2-dimethylhydrazine (DMH, 20 mg/kg), although crypt/ACF was high in the former relative to the latter. This result suggests that dietary wheat bran effectively serves as a regulator of ACF frequency at early stages after DMH injection.

Key words: aberrant crypt foci; 1,2-dimethylhydrazine; wheat bran; rat

Dietary factors, e.g., dietary fat, fiber, and several micronutrients, are associated with colorectal tumorigenesis. A high-fiber diet might diminish the risk of developing colorectal cancer but the mechanism is still unclear.

In rodents exposed to chemical carcinogens, aberrant crypt foci (ACF) and tumors develop in rat colon, reportedly. ACF appear at an early period of colorectal tumorigenesis as well as in the tumor-bearing state in rats after treatment with carcinogen and also in patients with sporadic and hereditary colon cancer. Aberrant crypts are a few times larger than normal crypts, occurring as oval shape in the thickened epithelium and are easily detectable in whole mounts of the colonic mucosa with methylene blue staining. They can therefore be considered as putative early preneoplastic lesions. On the other hand, there are complicated results that ACF formation is inhibited by administration of cholic acid which may promote colon tumors. It is interesting to investigate the effect of dietary factors on the growth characteristics of ACF to evaluate the biological significance of ACF or to discover the mechanism of tumor formation thereafter.

The aim of this study is to examine whether dietary wheat bran fiber modifies the early appearance of ACF induced by 1,2-dimethylhydrazine (DMH).

Five-week-old male Wister ST rats (Japan SLC Inc., Hamamatsu, Japan) were housed in individual cages in a temperature-controlled (23 ± 2°C) room with a 12 h photoperiod. The study was approved by the Hokkaido University Animal Use Committee, and animals were maintained according to the guidelines for the care and use of laboratory animals, Hokkaido University. The rats were acclimated to a fiber-free diet for 7 days and then given either the fiber-free diet or the diet with 10, 15, or 30% wheat bran (Wb). After 4 days, they were subcutaneously injected with 1 ml of DMH (20 mg/kg body weight) solution as described before. Rats from each group were killed by exsanguination under anesthesia with sodium pentobarbital (Abbott Laboratories, North Chicago, IL), 1, 2, 3, or 4 weeks after receiving the single injection of DMH. The colon was removed from the anal verge to proximal position of colon (about 3 cm from ileocecal valve

along the mesenteric side), flushed with cold saline, and divided into four portions with nearly the same length. From proximal to anal verge, the four colon portions were termed proximal colon (PC), middle colon (MC), distal colon (DC), and rectum (RT). Number of aberrant crypts and ACF in colon mucosa were counted as described by Bird. Butyric acid was extracted according to the method of Hara and analyzed using a Shimadzu GC-9A gas chromatograph with a flame ionization detector and 2.1 m x 3 mm column containing 10% FAL-M on Shimulte TPA (60-80 mesh). 2-Methylvaleric acid was added to the samples before extraction as an internal standard.

Comparisons for all pairs using Tukey–Kramer HSD were used to decide if there was any difference between groups. All statistical calculations were done using JMP computer software (SAS Institute Inc., NC). A probability less than 0.05 was considered significant.

The body weight and food intake were compared among rats fed the fiber-free diet or Wb-supplemented diets, there were no significant differences among the groups (data not shown).

After only a single injection of DMH, ACF were found at 2 weeks in the whole colon except PC (data not shown). The ACF count rapidly increased between 3 and 4 weeks in every portion of the colon after the injection of DMH, and the ACF count in DC was much higher than in PC, MC, or RT (data not shown). Such a rapid increase in ACF was observed for every portion of the colon in both groups, indicating that some tumorigenesis-promoting event occurred between 3 and 4 weeks after the injection of DMH. The formation of ACF was influenced by various chemicals, dietary components, and intestinal bacteria. The distribution of ACF was not affected by the 10% Wb in the diet.

Because ACF count was the highest in DC at 4 weeks after the

**Fig. 1. Number of ACF in DC at 4 Weeks after a Single Injection of DMH (20 mg/kg Body Weight).**

Columns not sharing common superscript letters are significantly different at $p < 0.05$ ($n=4-6$).

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Abbreviations: ACF, aberrant crypt foci; DMH, 1,2-dimethylhydrazine; Wb, wheat bran; PC, proximal colon; MC, middle colon; DC, distal colon; RT, rectum.
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Figure 3 shows the butyric acid concentration in the cecal contents at 4 weeks after the treatment with DMH. The butyric acid concentration was highly dependent on the increase in dietary Wb level. The ACF count was greatly reduced in DC, while the cecal butyric acid content was above 20 μmol/g cecum. The concentration of butyric acid did not change over a period of 4 weeks after the DMH treatment (data not shown). Butyric acid has been reported to induce apoptosis in some colon carcinoma cell lines by its addition at a physiological concentration.17) Butyric acid may reduce the formation of ACF by inducing apoptosis of the cells damaged by DMH.

In conclusion, it is highly possible that dietary Wb suppresses the frequency of colonic ACF induced by DMH.

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References