Food Deprivation Increases Apoptotic Cell Counts Induced by 1,2-Dimethylhydrazine in Rat Descending Colonic and Rectal Crypts

Satoshi Ishizuka, Kei Sonoyama, and Ryoya Niki

Laboratory of Food Biochemistry, Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

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Summary The frequency of apoptosis after treatment with 1,2-dimethylhydrazine (DMH) was counted in the descending colonic and rectal crypts of food-deprived and fed rats. Food-deprived or fed rats were subcutaneously injected with DMH (100 mg/kg body weight). Six hours after the injection, apoptotic cells were observed in crypt regions by light microscopy. The incidence of DMH-induced apoptosis in food-deprived rats was significantly higher than in fed rats. The incidence appeared to be higher in descending colon than in rectum. PAS staining revealed that DMH treatment lowered mucin secretion in crypts, which was substantially lowered by food deprivation. The effect of food deprivation on apoptosis induced by DMH may be due to the decrease in mucus barrier against DMH.

Key Words apoptosis, dimethylhydrazine, crypt, descending colon, rectum, colorectal cancer, rat

A colonic carcinogen, 1,2-dimethylhydrazine (DMH) is known to produce tumors of the lower colon even after a single dose (1), and a number of investigations have been performed to determine how dietary components (e.g. dietary fiber) influence the tumorigenesis of this agent (2-8). Ijiri (9) showed that the administration of DMH induced apoptosis mainly in the crypt cells of the lower part of the large bowel. Apoptosis is programmed cell death with morphological characteristics involving DNA fragmentation and nuclear condensation (10). The DMH-induced apoptosis in the crypt cells of the lower part of colon may be related to the carcinogenicity of this agent. It remains unclear, however, whether the dietary component or feeding status influence the apoptotic incidence induced by DMH. In this study, we have investigated the effect of food deprivation on DMH-induced apoptotic cell count in the crypts of the lower part of the colon in rats.

Materials and methods. Animals and diet. Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), 7 weeks old at the start of the experiment, were housed
Table 1. Composition of diet.

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>g/kg diet</th>
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<tbody>
<tr>
<td>Casein(^1)</td>
<td>250</td>
</tr>
<tr>
<td>Sucrose</td>
<td>647</td>
</tr>
<tr>
<td>Corn oil(^2)</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture(^3)</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mixture(^4)</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin E(^5)</td>
<td>1</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^1\) Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).  
\(^2\) Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg diet) were added to corn oil.  
\(^3\) The mineral mixture is identical with MM2 described by Ebihara et al. (14).  
\(^4\) The vitamin mixture was prepared in accordance with the AIN-76 mixture (15), except that vitamin K as menadione and L-ascorbic acid were added to give 5.81 μmol/kg (16) and 284 μmol/kg (17) of diet, respectively.  
\(^5\) Vitamin E (granulated, Juvela, Eisai Co., Tokyo, Japan) supplied 423 μmol all-rac-a-tocopheryl acetate/kg diet.

in individual cages in a temperature-controlled (23±2°C) room under a 12-h light/dark cycle (light 8:00–20:00 h). They were fed a standard diet (Table 1) ad libitum for 10 days and allowed free access to water. The rats weighing 188±5 g (n = 12) were divided into two groups: one group was deprived of food overnight, and the other was continued feeding before injection of 1,2-DMH (Aldrich Chemical Co. Inc., Milwaukee, Wis.). Each group of rats was subcutaneously injected with or without DMH (100 mg/kg body weight) in 1.5 mM EDTA saline, pH 6.5. The injection was performed between 9:00 and 10:00. Six hours after the injection, rats were sacrificed by exsanguination under anesthesia with 35 mg/kg body weight sodium pentobarbital (Abbott Laboratories, North Chicago, IL). The descending colon (DC) and rectum (RT), correspond to the sections 0 to 2 cm and 2 to 5 cm, respectively, from the anal verge, were excised and washed with phosphate-buffered saline (PBS). The samples were subjected to histological analysis.

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.

**Histological procedure.** The DC and RT were fixed in periodate lysine paraformaldehyde solution for 4 h, thereafter they were immersed in 30% sucrose in PBS overnight. After washing with PBS, samples were frozen at −80°C. Frozen sections (4 μm) were prepared with a cryostat (MINOTOME, International Equipment Co., Boston, MA) and stained with hematoxylin and eosin for apoptotic cell counting. Mucin in the colonic surface mucous layer was detected by periodic acid-Schiff (PAS) staining and counterstaining with hematoxylin. The slide glasses were coated with casein and BSA beforehand.

Scoring of apoptotic cells. DMH-induced apoptosis was counted by a light microscope according to the method of Ijiri (9). When several apoptotic nuclear fragments were observed, they were recorded as a single apoptotic cell. The number of epithelial cells per one side of crypt section were also counted from the base of the crypt to the top. Twenty-five crypt sections were scored for each rat.

Statistical analysis. Student's t-test was used to determine whether mean values were significantly different. Values in the text are M±SEM.

Results. Apoptotic cells in crypt sections following treatment with 100 mg/kg body weight DMH were morphologically identified under light microscope by nuclear condensation and apoptotic bodies, which were clearly observed (Fig. 1). Therefore, it could be discriminated morphologically from mitosis. In longitudinal section, these apoptotic cells were observed in the lower part of the crypt.

In DC (Fig. 2A), the apoptotic cell counts were significantly increased with DMH injection in both the food-deprived and fed groups. The incidence of apoptosis in the DMH-injected animals was significantly higher in food-deprived group than in fed group. Similar results were obtained in RT (Fig. 2B), but there was no significant difference in apoptosis between food-deprived and fed rats injected with DMH. The incidence of DMH-induced apoptosis seemed to be higher.

Fig. 1. Longitudinal crypt section prepared from rat DC following treatment with 100 mg/kg DMH s.c. Apoptotic cells and nuclear fragments can be seen in the lower part of the crypt. The sections were stained with hematoxylin and eosin (bar, 10μm).
Fig. 2. Apoptotic cell counts per crypt section following treatment with DMH (■) or the vehicle (□) in DC (A) and RT (B). Data and error bars are expressed as the mean value of 3 rats±SEM. NS means no significant difference.

in DC than RT. The total number of epithelial cells per one side of the crypt sections in DC were not significantly different among all groups (Fast DMH, 45.3 ± 1.2; Fast vehicle, 45.2 ± 1.1; Fed DMH, 46.2 ± 1.5; Fed vehicle, 45.7 ± 0.9) and this was also in the case for RT (Fast DMH, 42.3 ± 1.5; Fast vehicle, 42.7 ± 1.0; Fed DMH, 43.7 ± 0.8; Fed vehicle, 43.2 ± 0.1).

Figure 3 shows the crypt sections stained with PAS. In the case of vehicle injection, PAS-positive granules and mucous coatings were not different between food-deprived (Fig. 3A) and fed (Fig. 3B) animals. In contrast, in the case of DMH injection, mucin contents in the lower part of the crypts were significantly decreased in food-deprived rats (Fig. 3C) compared to those in fed rats (Fig. 3D). Similar results were obtained in RT (data not shown).

Discussion. Cancer in the gastrointestinal tract occurs in characteristic locations. Very few cancers appear in the small intestine, but there is a high incidence...
in the large bowel. DMH is converted into azoxymethane (AOM) or methylazoxymethanol-β-glucuronide (M-β-G) in the liver. AOM and M-β-G in bile juice are excreted into the intestinal lumen. Subsequently, intestinal bacteria convert AOM and M-β-G into a carcinogen, methylazoxymethanol (MAM) (11). MAM is absorbed by enterocytes. There is an interesting report showing that apoptosis is induced by carcinogens (e.g. DMH) and radiation in the crypt at the colon where the incidence of spontaneous cancer is higher (9). Apoptosis is a mode of cell death involving nuclear condensation and fragmentation (10). In the gastrointestinal tract, apoptosis has usually been shown to occur at the top of the villus (12). Apoptotic cells, however, appeared to be in the crypt base after treatment with
DMH injection (13). Stem cells are located at the lower part of the crypt. Furthermore, the carcinogenesis is generally recognized as the result of mutation of stem cells.

Many studies have reported on the effects of dietary treatment on DMH-induced carcinogenesis (2–8), but it is not known whether dietary component or feeding status influence DMH-induced apoptosis or not. In this study, we have investigated the effect of food deprivation on apoptosis induced by DMH.

The dosage of DMH (100 mg/kg) is enough to induce colon tumors, because a single dose of DMH (35 mg/kg) induces colon carcinoma (1). Ijiri (9) reported a steady increase in apoptotic cells after treatment with DMH, and that this increase continued from 3 to 12 h. In this study, the sampling of DC and RT was performed 6 h after DMH injection. Consequently, many apoptotic cells were observed in DC and RT of rats injected with DMH. Especially in DC, the numbers of DMH-induced apoptosis counts were significantly higher in food-deprived rats than in fed rats.

The luminal surface of epithelial cells is covered with mucin secreted from goblet cells as a physical barrier. In this study, we observed that DMH decreased mucin granules in the lower part of crypt in food-deprived rats. Under food-deprivation, treatment with DMH may repress the mucin production in goblet cells. Consequently, the decrease in mucous barrier may increase the chance of exposing the stem cells to the carcinogen.

In conclusion, our results suggest that the increase in DMH-induced apoptosis in DC crypts induced by food deprivation may be due to the decrease in the mucosal barrier. To understand the relationship between colon carcinogenesis and dietary components, it is important to study the mechanism by which dietary components influence DMH-induced apoptosis.

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REFERENCES


FOOD DEPRIVATION INCREASES COLONIC APOPTOSIS INDUCED BY DMH

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