Modifying effects of a flavonoid morin on azoxymethane-induced large bowel tumorigenesis in rats

Takuji Tanaka6, Kunihiro Kawabata2, Mikio Kakumoto4, Hiroki Makita3, Jun Ushida2, Shiro Honjo1, Akira Hara4, Hiroyuki Tsuda5 and Hideki Mori2

Introduction

Colorectal cancer is the third most malignant neoplasm in the world. It is the second leading cause of cancer deaths in the USA and the third in Japan. In Japan, the progressive introduction of Western dietary habits, especially an increasing fat intake and decreasing carbohydrate intake, has increased the incidence of colorectal cancer (1). Although the causes of colorectal cancer are not completely understood, dietary factors appear to be important for the development of colorectal cancer (2).

Dietary factors play an important role in human health and in the development of certain chronic diseases, including cancer (3,4). Some foods contain macro- and micronutrients with antitumorogenic effects as well as mutagens and/or carcinogens (5). Active primary prevention, including chemoprevention, against cancer development is now important and many studies are currently directed at identifying possible chemopreventive agents (6), especially those that are naturally found in foods. In a large number of epidemiological data, a protective effect of the consumption of vegetables and fruits against various forms of cancer was found (7–9). In studies of individual subjects, high levels of consumption of vegetables and fruits are consistently associated with a low risk of colorectal cancer (2,8). Possible explanations for these findings are that people who eat more vegetables and fruits avoid possible carcinogens in meats and fats (10) and that vegetables and fruits contain anticarcinogens that block the development of colorectal neoplasms. Many natural compounds in vegetables and fruits prevent cancer development in experiments with animals (11).

Our group has reported several possible chemopreventive agents in edible plants, including fruits and vegetables, against large bowel carcinogenesis (12–14). A high consumption of fruits and green/yellow vegetables has consistently been found to be associated with a low incidence of many types of cancer, since Hirayama drew attention to the relationship in his cohort study of 270 000 Japanese in 1979 (15). Thus, non-nutritive constituents such as flavonoids in fruits and vegetables may contribute to reduced risk of cancer occurrence. Among compounds of known structure, flavonoids deserve special attention because they are present in practically all dietary plants, fruits and roots. They are consumed daily in considerable amounts (at least 23 mg; 16) and are heat stable. Additionally, the flavonoids, including morin (17,18), are nontoxic (19,20) and display a variety of biological actions, such as anti-allergic, anti-inflammatory, scavenging of free radicals, modulation of enzymatic activities, antigenerative and antitumorogenic, to varying degrees (21–23). Thus, flavonoids are considered as candidates for chemopreventive or therapeutic agents against free radical-associated diseases. Recent studies in our laboratory have suggested that two flavonoids, diosmin and hesperidin, exert effective cancer protective actions on oral, esophageal, large bowel and urinary bladder carcinogenesis in rats (12,24–27). Morin (3,5,7,2’;4’-pentahydroxyflavone; Figure 1) is a kind of flavonoid found in the fig and other Moraceae which are used as herbal medicines. It has certain biological activities, including antioxidant properties (28,29) and a modulatory effect on lipoxygenase (LOX) and cyclooxygenase (COX) activities in the arachidonic cascade (30,31). Inhibitors of prostaglandin biosynthesis are known to inhibit colon carcinogenesis (32). Morin also acts as an antimutagen.

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Morin has been reported to have an antipromotion activity in a liver carcinogenesis model (36). Several flavonoids, including morin, inhibit activation of protein kinase C by teleocidin in mouse skin (37). These findings led us to investigate possible modulatory (inhibitory) effects on rat colorectal carcinogenesis, although the morin content in foods is lower than the major flavonoid quercetin (16).

In the present study, a potential chemopreventive effect of morin on colorectal carcinogenesis initiated with azoxymethane (AOM) was investigated in male F344 rats by determining the incidences of colorectal neoplasms. The effect of morin on the development of preneoplastic lesions, colorectal aberrant crypt foci (ACF) (38), was also evaluated. As for the mechanistic investigation, the effect of dietary morin on the cell proliferation activity of colorectal mucosal epithelium was assessed by measuring the proliferating cell nuclear antigen (PCNA)-positive index. Also, tissue (colorectal mucosa) and blood polyamine levels were assayed as a biochemical biomarker for cell proliferation. In addition, glutathione S-transferase (GST) and quinone reductase (QR) were assayed in the liver and colorectal mucosa to determine whether morin could affect tumor incidence via modification of these enzymes.

Materials and methods

Animals, diets and carcinogen

Male F344 rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) aged 4 weeks were used. All animals were housed in wire cages (three or four rats per cage) with free access to drinking water and basal diet (CE-2; Clea Japan, Tokyo, Japan), under controlled conditions of humidity (50 ± 10%), lighting (12 h light/dark cycle) and temperature (23 ± 2°C). They were quarantined for 14 days and randomized into experimental and control groups. Powdered CE-2 diet (345-2 cal) was used as basal diet throughout the study. It contained 45.5% crude carbohydrate, 24.8% crude protein, 4.6% crude fat, 7.2% ash, 4.2% crude cellulose, 3.9% minerals, 1% vitamin mixture and 8.8% water. Morin was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan) and the experimental diet was made by mixing morin at a concentration of 500 p.p.m. The concentration of morin in the diet was 1/10 of the dose which did not show any toxic effects in rats (17). The experimental diet was stored in a cold room (4°C) until used. The diet and drinking water were freely available during the study. AOM was obtained from Sigma Chemical Co. (St Louis, MO). AOM (15 mg/kg body wt) was given by s.c. injection between 10:00 and 11:00 a.m. to produced colorectal ACF and neoplasms.

Experimental procedure

The experiment was designed to examine the modifying effect of morin during the initiation and post-initiation phases of AOM-induced large bowel tumorigenesis in male F344 rats. A total of 55 male F344 rats were divided into five groups as shown in Figure 2. Groups 1–3 were initiated with AOM by three weekly s.c. injections (15 mg/kg body wt). Rats in group 2 were fed the diet containing 500 p.p.m. morin for 4 weeks, starting 1 week before the first dosing of AOM. They were then switched to the basal diet and maintained on this diet for 28 weeks. Group 3 was fed the diet mixed with 500 p.p.m. morin for 28 weeks, starting 1 week after the last injection of AOM. Group 4 was given the diet containing 500 p.p.m. morin during the study. Group 5 served as an untreated control. When the experiment was terminated, all animals were killed to assay the incidences of ACF and neoplasms in the large bowel. After fixation in 10% buffered formalin, the frequency of preneoplastic lesions was determined and number and size of oral and large bowel tumors were recorded. Tissues were embedded in paraffin blocks and processed for routine histological observation with hematoxylin and eosin stain.

Identification of ACF

At the end of the study, the number of ACF in the colorectal mucosa was counted in seven rats in group 1, nine rats in group 2, nine rats in group 3, five rats in group 4 and four rats in group 5. After fixation for at least 24 h at room temperature, large bowels were stained in 0.5% methylene blue (in saline) for 1–3 min. After staining, they were examined for ACF by light microscopy at 40× magnification and ACF were recorded according to the procedure described by Bird (39) and Tanaka et al. (13). In this study, we divided the colon into three portions (proximal, middle and distal) and counted ACF in each portion.

PCNA immunohistochemistry

PCNA-positive cell nuclei were counted in the colorectal mucosa (ACF and ‘normal-appearing’ colorectal mucosa) of seven rats in group 1, nine rats in group 2, nine rats in group 3, five rats in group 4 and four rats in group 5. Anti-PCNA antibody (Dako Co., Kyoto, Japan) was used with the avidin–biotin complex method. Immunohistochemical staining was performed according to the method in our previous paper (40). Tissue sections were deparaffinized with xylene, hydrated through a graded ethanol series and incubated with 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. They were then incubated with 10% normal horse serum at room temperature for 30 min to block background staining and then incubated with 100 µl anti-PCNA antibody (dilution 1:300) at room temperature for 1 h. ACF and normal-appearing crypts were also used to determine the PCNA-positive index in the large bowel. The total number of cells in each crypt was calculated by adding the number of PCNA-positive and PCNA-negative cells. The PCNA-positive index was determined by dividing the number of PCNA-positive cells by the total number of cells in the crypt×100.

Assay of tissue and blood polyamine levels

At autopsy, tissue and blood polyamine levels were measured in five rats each of groups 1–3 and three rats each of groups 4 and 5. The colorectal mucosa was immediately scraped for measurement of tissue polyamine levels. Blood samples (5 ml/rat) were collected by heart puncture. Tissue and blood polyamine levels were determined by the method of Koido et al. (41).

Measurement of GST and QR activities in the liver and colorectal mucosa

GST and QR activities of liver and colorectal mucosa were determined from tissues (mainly in the middle and distal colon) and some in the small intestine of rats in groups 1–3. No neoplasms were found in any organs of rats in groups 4 and 5. These sessile or...
Modulation of large bowel tumorigenesis by morin

Fig. 2. Experimental protocol.

Table I. Incidence of intestinal neoplasms of rats fed morin during or after AOM exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Rats examined</th>
<th>Rats with neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Entire intestine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>12</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>2</td>
<td>AOM + 500 p.p.m. morin</td>
<td>14</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>3</td>
<td>AOM → 500 p.p.m. morin</td>
<td>14</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>4</td>
<td>500 p.p.m. morin</td>
<td>8</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>5</td>
<td>No treatment</td>
<td>7</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*AD, adenoma; ADC, adenocarcinoma.

Statistically different from group 1 by Fisher’s exact probability test ($P = 0.023$).

Pedunculated colorectal tumors were histologically tubular adenomas and adenocarcinomas, including signet-ring cell carcinomas, with a higher incidence of adenocarcinoma. A few rats had renal mesenchymal tumors and/or altered hepatocellular foci in groups 1–3, but these lesions were not found in other groups. The incidence and multiplicity of intestinal neoplasms are shown in Tables I and II, respectively. The incidence of small intestinal adenocarcinoma in group 2 (morin feeding together with AOM administration) was larger than that in group 1 (AOM alone). In contrast, the incidence of large intestinal adenocarcinoma in group 2 was smaller than that in group 1. However, the differences in these incidences were not statistically significant. The incidences of entire intestinal tumors in rats of groups 1 and 2 were almost comparable. The incidence (29%) of large intestinal adenocarcinoma in group 3 (morin feeding after AOM administration) was significantly smaller than that of group 1 (75%) ($P = 0.023$), although the differences in the incidences of entire intestinal tumors between these two groups were not statistically significant. As shown in Table II, multiplicities of large intestinal adenocarcinoma in groups 2 and 3 were lower than that in group 1, but this was not statistically significant. However, the multiplicity of adenocarcinomas in the entire intestine (small and large intestine) in group 3 (morin feeding after AOM
Table II. Multiplicity of intestinal neoplasms of rats fed morin during or after AOM exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Rats examined</th>
<th>Rats with neoplasms</th>
<th>Entire intestine</th>
<th>Small intestine</th>
<th>Large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total AD</td>
<td>Total AD</td>
<td>Total AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AD ADC</td>
<td>AD ADC</td>
<td>AD ADC</td>
</tr>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>12</td>
<td></td>
<td>1.33 ± 0.17</td>
<td>1.17 ± 0.33</td>
<td>0.33 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.94 ± 0.37</td>
<td>0.80 ± 0.62</td>
<td>0.62 ± 0.62</td>
</tr>
<tr>
<td>2</td>
<td>AOM + 500 p.p.m. morin</td>
<td>14</td>
<td></td>
<td>1.36 ± 0.21</td>
<td>1.07 ± 0.57</td>
<td>0.57 ± 0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.11 ± 0.41</td>
<td>0.96 ± 0.73</td>
<td>0.73 ± 0.73</td>
</tr>
<tr>
<td>3</td>
<td>AOM → 500 p.p.m. morin</td>
<td>14</td>
<td></td>
<td>0.71 ± 0.14</td>
<td>0.57 ± 0.21</td>
<td>0.21 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.59 ± 0.35</td>
<td>0.49 ± 0.41</td>
<td>0.41 ± 0.41</td>
</tr>
</tbody>
</table>

*AD, adenoma; ADC, adenocarcinoma.
*Mean ± SD.
*Statistically different from group 1 by Welch’s t-test (P < 0.05).

Table III. Incidence of ACF at the end of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Incidence</th>
<th>ACF/colon</th>
<th>ACF/cm²</th>
<th>Aberrant crypts/colon</th>
<th>Aberrant crypts/focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>12/12</td>
<td>104 ± 33a</td>
<td>7.85 ± 1.67</td>
<td>220 ± 48</td>
<td>2.11 ± 0.32</td>
</tr>
<tr>
<td>2</td>
<td>AOM + 500 p.p.m. morin</td>
<td>14/14</td>
<td>52 ± 7b</td>
<td>4.17 ± 0.79b</td>
<td>113 ± 20b</td>
<td>2.20 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>AOM → 500 p.p.m. morin</td>
<td>14/14</td>
<td>50 ± 12b</td>
<td>3.19 ± 1.17b</td>
<td>118 ± 32b</td>
<td>2.37 ± 0.38</td>
</tr>
<tr>
<td>4</td>
<td>500 p.p.m. morin</td>
<td>0/8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>No treatment</td>
<td>0/7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean ± SD.
*Significantly different from group 1 by Welch’s t-test or Student’s t-test (P < 0.001).

Table IV. PCNA labeling index (%) of ACF and surrounding normal-appearing colonic crypts

<table>
<thead>
<tr>
<th>Group</th>
<th>ACF</th>
<th>Normal-appearing crypts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (AOM alone)</td>
<td>27.9 ± 6.3a</td>
<td>18.7 ± 4.4</td>
</tr>
<tr>
<td>Group 2 (AOM + 500 p.p.m. morin)</td>
<td>20.3 ± 4.6</td>
<td>16.9 ± 4.2</td>
</tr>
<tr>
<td>Group 3 (AOM → 500 p.p.m. morin)</td>
<td>18.8 ± 5.2b</td>
<td>14.9 ± 4.9</td>
</tr>
<tr>
<td>Group 4 (500 p.p.m. morin)</td>
<td>14.2 ± 4.2</td>
<td>15.1 ± 3.4</td>
</tr>
<tr>
<td>Group 5 (No treatment)</td>
<td>14.2 ± 4.2</td>
<td>15.1 ± 3.4</td>
</tr>
</tbody>
</table>

*Mean ± SD. Data are from five rats from each group.
*Significantly different from group 1 by Student’s t-test (P < 0.05).

administration) was significantly lower than that in group 1 (P < 0.05).

Frequency of ACF at the end of the study
The incidence of ACF is shown in Table III. In the long-term study, ACF developed in rats treated with AOM, with or without morin feeding (groups 1–3), while no ACF were present in groups 4 and 5. The frequencies of ACF per large bowel, the number of ACF per area (cm²) and the number of aberrant crypts per colorectum in groups 2 and 3 were significantly lower than those in group 1 (P < 0.001). However, there were no significant differences in the number of aberrant crypts per focus among the groups.

PCNA-positive index
As shown in Table IV, the PCNA labeling index in ACF was generally greater than in normal-appearing crypts. Morin feeding reduced the PCAN labeling index in both ACF and normal-appearing crypts. The PCNA index in ACF of group 3 was significantly smaller when compared with that of group 1 (P < 0.05).

Polyamine levels in colorectal mucosa and blood
The results of the large bowel and blood polyamine assays are shown in Tables V and VI, respectively. AOM treatment significantly elevated polyamine levels in the colorectal mucosa and blood (P < 0.001 and P < 0.02). Morin feeding significantly decreased the mucosal polyamine content (P < 0.02 and P < 0.005; Table V) and blood polyamine levels (P < 0.05; Table VI). Spermine concentrations in colonic mucosa of rats treated with morin during or after AOM administration significantly increased when compared with the AOM alone group (P < 0.001 and P < 0.005; Table V). Also, morin feeding (group 4) significantly increased mucosal spermine content compared with the untreated control (group 5) (P < 0.02). In blood, spermine concentrations of rats treated with morin during or after AOM administration decreased when
compared with the AOM alone group (Table VI). Feeding of morin (group 4) significantly decreased blood spermine levels when compared with the untreated group (group 5) \((P < 0.01)\).

**GST and QR activities in the colorectal mucosa**

The results for GST and QR activities of the large bowel mucosa are presented in Table VII. AOM treatment significantly reduced GST and QR activities in the liver and large bowel \((P < 0.001)\) and \((P < 0.01)\). Morin feeding significantly increased colorectal GST activity in groups 2 and 3 \((P < 0.05, P < 0.001)\). The increases in the activities of GST and QR in the liver of rats in group 2 were not statistically significant when compared with those of group 1.

Also, dietary morin significantly increased colorectal QR activity in group 4, when compared with group 5 \((P < 0.02)\).

**Discussion**

Dietary feeding of morin during the post-initiation phase significantly lowered the incidence of large bowel adenocarcinoma induced by AOM, but not the multiplicity of adenocarcinoma. Feeding of morin during the initiation phase did not alter the incidence and multiplicity of intestinal neoplasms. These results indicate that the inhibitory effect of morin on AOM-induced colorectal carcinogenesis was relatively weak. In the present study, feeding a morin-containing diet at a dose level

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**Table V.** Tissue polyamine levels in colonic mucosa of rats treated with AOM and/or morin

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (no. of rats)</th>
<th>Polyamine levels (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diamine</td>
</tr>
<tr>
<td>1</td>
<td>AOM alone (5)</td>
<td>0.7 ± 1.1a</td>
</tr>
<tr>
<td>2</td>
<td>AOM + 500 p.p.m. morin (5)</td>
<td>0.6 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>AOM → 500 p.p.m. morin (5)</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>500 p.p.m. morin (3)</td>
<td>1.1 ± 1.6</td>
</tr>
<tr>
<td>5</td>
<td>No treatment (3)</td>
<td>0.6 ± 0.6</td>
</tr>
</tbody>
</table>

a,b Mean ± SD.
c,d Significantly different from group 5 by Student’s \(t\)-test \((P < 0.01)\) and \(t\)-test \((P < 0.001)\).
d,e Significantly different from group 1 by Student’s \(t\)-test \((P < 0.001)\) and \(t\)-test \((P < 0.02)\) and \(t\)-test \((P < 0.005)\).

**Table VI.** Blood polyamine levels in rats treated with AOM and/or morin

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (no. of rats)</th>
<th>Polyamine levels (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diamine</td>
</tr>
<tr>
<td>1</td>
<td>AOM alone (5)</td>
<td>0.3 ± 0.3a</td>
</tr>
<tr>
<td>2</td>
<td>AOM + 500 p.p.m. morin (5)</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>AOM → 500 p.p.m. morin (5)</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>500 p.p.m. morin (3)</td>
<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>No treatment (3)</td>
<td>0.3 ± 0.5</td>
</tr>
</tbody>
</table>

a,b Mean ± SD.
c,d Significantly different from group 5 by Student’s \(t\)-test \((P < 0.02)\).
d,e Significantly different from group 1 by Student’s \(t\)-test \((P < 0.05)\).
e,f Significantly different from group 5 by Student’s \(t\)-test \((P < 0.01)\).

**Table VII.** GST and QR activities of colonic mucosa of rats treated with AOM and/or morin

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tissue</th>
<th>Group 1 (AOM alone) (Enzyme activity (mU/mg protein))</th>
<th>Group 2 (AOM + 500 p.p.m. morin) (Enzyme activity (mU/mg protein))</th>
<th>Group 3 (AOM → 500 p.p.m. morin) (Enzyme activity (mU/mg protein))</th>
<th>Group 4 (500 p.p.m. morin) (Enzyme activity (mU/mg protein))</th>
<th>Group 5 (No treatment) (Enzyme activity (mU/mg protein))</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST (CDNB)</td>
<td>Liver</td>
<td>517.3 ± 9.4b</td>
<td>538.3 ± 41.6</td>
<td>583.3 ± 53.3c</td>
<td>605.7 ± 24.7</td>
<td>585.7 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>72.6 ± 4.8b</td>
<td>100.9 ± 48d</td>
<td>113.9 ± 7.7d</td>
<td>115.7 ± 4.2</td>
<td>109.7 ± 7.2</td>
</tr>
<tr>
<td>QR (NADH)</td>
<td>Liver</td>
<td>51.7 ± 8.9f</td>
<td>65.7 ± 15.1</td>
<td>82.7 ± 14.4f</td>
<td>122.7 ± 43.7</td>
<td>132.7 ± 36.9</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>282.8 ± 37.3b</td>
<td>354.8 ± 54.8c</td>
<td>375.6 ± 28.5d</td>
<td>543.8 ± 25.2f</td>
<td>496.8 ± 23.4</td>
</tr>
</tbody>
</table>

a,b Mean ± SD.
c,d Significantly different from group 5 by Student’s \(t\)-test \((P < 0.001)\).
e,f Significantly different from group 1 by Student’s \(t\)-test \((P < 0.005)\) and \(t\)-test \((P < 0.001)\).
g Significantly different from group 5 by Student’s \(t\)-test \((P < 0.01)\).
h Significantly different from group 1 by Student’s \(t\)-test \((P < 0.005)\).
i Significantly different from group 5 by Student’s \(t\)-test \((P < 0.02)\).
of 500 p.p.m. did not cause a retardation of body weight gain. Also, no significant pathological alterations in the major organs (liver, kidney, lung, heart, etc.) were found in rats fed the diet containing morin during the study. These results indicate low or no toxicity of morin.

ACF, being preneoplastic lesions of colorectal carcinoma, are useful intermediate biomarkers to detect modifying effects of certain natural and synthetic compounds on chemically induced carcinogenesis. In the present study, morin feeding during either the initiation or post-initiation phase significantly reduced the occurrence of ACF at the end of the study, but a significant reduction in large bowel adenocarcinoma was only found when the morin-containing diet was fed during the post-initiation stage. Cell proliferation plays an important role in multistage carcinogenesis with multiple genetic changes (48,49). Several cellular components, such as polyanimes and polyamine synthetic enzyme activities, have been associated with cell proliferation. A decrease in the numbers of PCNA-positive cells reflects a decrease in S phase cells and thus reduced proliferative activity. Recent advances in molecular analysis of the cell cycle have revealed how fidelity is normally achieved by the coordinated activity of cyclin-dependent kinase, checkpoint controls and repair pathways and how this fidelity can be abrogated by specific genetic changes (50). These are present at high levels in proliferating normal and neoplastic tissues. Most of the possible chemopreventive agents against carcinogen-induced large bowel carcinogenesis suppress cell proliferation activity (14,51). We estimated cell proliferation using the PCNA-positive index (normal-appearing colorectal mucosa and ACF) and polyamine levels (large bowel mucosa and blood). PCNA-positive indices of ACF and normal-appearing mucosa in groups 2 and 3 were lower than those in group 1, but a significant difference for ACF was present between groups 1 and 3. The results on total polyamine levels of large bowel mucosa and blood indicate that morin feeding significantly lowered polyamine levels in groups 2 and 3 when compared with those in group 1. Treatment with AOM and morin significantly increased spermine concentrations in colonic mucosa when compared with AOM alone, presumably as a result of morin treatment alone. However, such an alteration was not observed in blood. The reason for this discrepancy is not known. Total polyamine level and/or spermidine/spermine ratio, but not a single polyamine (spermine), may reflect proliferation of colorectal mucosa, since there are no corresponding changes in the amount of spermine in hyperplasia and hypoplasia of intestinal mucosa (52). Also, changes in the two enzymes ornithine decarboxylase and diamine oxidase (53) might occur in the colorectal mucosa of rats treated with AOM and morin. In any case, our findings suggest that among cell proliferation biomarkers PCNA labeling index and total polyamine content of the colorectal mucosa are well correlated with the incidence of colorectal adenocarcinoma. Thus, one of the mechanisms of the chemopreventive activity of morin might be related to suppression of cell proliferation in ACF, especially when the compound is given during the post-initiation phase. Also, it is possible that post-initiation feeding of morin accelerated the disappearance of ACF with a dysplastic nature (14,54,55), which will progress colorectal cancer.

The metabolic activation of AOM by a form of cytochrome P450 (II1) to methyldioxymethanol, a proximate metabolite of AOM, occurs mainly in liver and colon (56). It is evident that the rates of liver metabolism of AOM and of methyldioxymethanol play a significant role in determining colon carcinogenicity. Several mechanisms by which chemopreventive agents exert their inhibitory effects on tumorigenesis have been suggested (57). Among them certain phase II detoxifying enzyme inducers are considered to be promising chemopreventive agents against cancer (58). It is not known whether morin could alter P450II1 activity, since in the current study we did not measure cytochrome P450II1 in the liver and colon. However, morin feeding affected the activities of the detoxifying enzymes GST and QR in the liver and colon. In group 2 ('initiation feeding'), significant elevations in GST and QR activities were seen in the colorectal mucosa but not in the liver. However, significant elevations in GST and QR activities occurred in both organs in group 3 ('post-initiation feeding'). Thus, feeding of morin during the initiation phase did not enhance the detoxifying enzymes enough to detoxify the ultimate carcinogen of AOM. The difference in the induction of detoxifying enzymes between feeding schedules may reflect the difference in the inhibitory effects of morin between groups 2 and 3. AOM was reported to cause oxidative stress in the colonic mucosa (59). Since morin has antioxidative properties (28,29), the cancer inhibitory effect of morin during the post-initiation stage may be due to modification of cell hyperproliferation in the colorectal mucosa exposed to AOM through suppression of oxidative stress (59).

Unexpectedly, the chemopreventive effect of morin on AOM-induced large bowel tumorigenesis was relatively weak and the effect was only found when morin was fed during the post-initiation phase. The reason for this is unknown. Since morin is a dual inhibitor of LOX and COX (30,31) and some COX or LOX inhibitors could modulate carcinogenesis (60), including colon carcinogenesis (32,61), we expected an inhibitory effect on large bowel tumorigenesis. However, the chemopreventive effect of morin on large bowel tumorigenesis was not remarkable in the current study. Recently, it has been reported that COX-2 levels are increased in colonic tumors in AOM-treated rats (62) and a selective COX-2 inhibitor effectively suppresses AOM-induced rat colon carcinogenesis (63). Thus, it may be possible that dietary morin at a dose of 500 p.p.m. did not significantly affect COX-2 level in the colorectal mucosa of rats in the current study. In addition, the pro-oxidant activity of morin (64) may result in its weak inhibition of large bowel tumorigenesis.

In conclusion, the results of our experiments show a weak chemopreventive effect of dietary morin on AOM-induced rat large bowel carcinogenesis by altering cell proliferation activity and/or detoxifying enzyme activities, when fed during the post-initiation phase. Additional studies on the modifying effect on COX-2 level in the colorectal mucosa exposed to AOM is on-going in our laboratories.

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