Chemopreventive effect of fermented brown rice and rice bran (FBRA) on the inflammation-related colorectal carcinogenesis in $Apc^{Min/+}$ mice

SUPHOT PHUTTHAPHADOONG$^{1,2}$, YASUHIRO YAMADA$^1$, AKIHIRO HIRATA$^3$, HIROYUKI TOMITA$^1$, AKIRA HARA$^1$, PORNNGARM LIMTRAKUL$^2$, TERUAKI IWASAKI$^4$, HIROSHI KOBAYASHI$^5$ and HIDEKI MORI$^1$

$^1$Department of Tumor Pathology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan; $^2$Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand; $^3$Division of Animal Experiment, Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193; $^4$Genmai Koso Co., Ltd., Nishi 1-chome, Kita 12-jo, Kita-ku, Sapporo 001-0012; $^5$Sapporo Cancer Seminar, 6 Odori-Nishi, Chuo-ku, Sapporo 064-0820, Japan

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Abstract. Our previous study revealed that fermented brown rice and rice bran (FBRA) suppresses rat colorectal carcinogenesis induced by azoxymethane, the colon-specific carcinogen. In the present study, we examined the suppressive effect of FBRA on colon carcinogenesis in $Apc^{Min+}$ mouse, a mouse model for human familial adenomatous polyposis. In contrast to previous findings with the carcinogen-induced model, administration of 5 and 10% FBRA had no effect on the tumor development in the colon of $Apc^{Min+}$ mice, suggesting that the modifying effects of FBRA on colorectal carcinogenesis are different depending on rodent models for colorectal carcinogenesis. However, when FBRA is administrated in dextran sodium sulfate (DSS)-exposed $Apc^{Min+}$ mouse, a mouse model for the inflammation-related colorectal carcinogenesis, FBRA significantly suppressed the multiplicity of colon tumors in comparison with control diet group. FBRA administration suppressed the cell proliferative index, which is accompanied by the significantly decreased mRNA expressions of Cox2 and iNos in colonic mucosa exposed to DSS (p<0.04 and 0.02, respectively). These findings indicate that FBRA has chemopreventive effects specifically against inflammation-related tumorigenesis in the colon. Our findings also suggest that anti-inflammatory activity is one of the underlying mechanisms by which FBRA suppresses tumorigenesis in the colon.

Introduction

Inflammation has been recognized as a major factor in the pathogenesis of several common diseases, including cancer. Patients with inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), have an increased risk of colorectal cancer (CRC) (1). The molecular alterations in colitis-associated CRC are thought to be similar to the adenoma-carcinoma sequence in sporadic colorectal carcinoma, including the loss of function of the APC, p53 and K-ras gene. However, it is suggested that the timing and frequency of these genetic alterations in colitis-associated CRC occur differently from sporadic colorectal carcinomas (2-4).

Animal models of experimental colitis have been developed and are often used to evaluate new treatments for IBD (4). As the onset of inflammation is immediate and the procedure is relatively straightforward, chemically induced models of intestinal inflammation, such as the dextran sodium sulfate (DSS) model, belong to the most commonly used IBD animal models (5,6). Importantly, when $Apc^{Min+}$ mice, which harbor a germline mutation in the $Apc$ gene, are exposed to DSS, colitis markedly accelerates the development of dysplasia and cancer in the colon of $Apc^{Min+}$ mice (7). Therefore, it is now regarded that DSS-treated $Apc^{Min+}$ mouse is one of rodent models for inflammation-related colorectal tumorigenesis.

Epidemiological studies and experimental evidence have suggested a potential relationship between components of the diet and several diseases, including IBD and cancer (8-10). A large number of experimental studies have shown that high intake of dietary fiber, vegetables, fruits decreases the incidence of colorectal cancer (11). Fermented brown rice and rice bran (FBRA) is a processed food prepared by fermented the brown rice and rice bran with Aspergillus oryzae. In our previous studies, FBRA showed chemopreventive effect against various types of chemically-induced cancer, including colon (12), stomach (13), bladder (14) and esophagus (15) in rodent models. In addition, FBRA has a suppressive effect on
the acute colitis in rats administered DSS (16). In the present study, we examined the chemopreventive activity of FBRA on colon tumorigenesis in ApclMin/+ mice, a genetic model for colon carcinogenesis, as well as inflammation-related colorectal carcinogenesis using ApclMin/+ mice administered DSS.

Materials and methods

Animals. The ApclMin/+ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The ApclMin/+ pedigree was maintained by mating C57BL/6J females with ApclMin/+ males. The ApclMin/+ mice were identified by allele-specific PCR on DNA isolated from the mouse tail. Male C57BL/6J mice (4 weeks of age) were purchased from SLC (Hamamatsu, Japan). All mice were maintained under specific pathogen-free conditions with isolated ventilation cages in an air-conditioned room with a 12:12 L:D cycle, with free access to water and were bred and maintained on a basal diet, CE-2 (CLEA Japan Inc., Tokyo, Japan).

Chemicals. Powdered CE-2 diet was used as the basal diet throughout the study. The experimental diets were prepared by mixing 5.0 and 10.0% FBRA with CE-2 diet. FBRA was supplied by Genmai Koso Co., Ltd. (Sapporo, Japan). Briefly, the manufacturing process of FBRA was: fermentation base was made by steaming of brown rice and rice bran. Aspergillus oryzae was then seeded to the fermentation base and fermentation process was continued for 18-24 h. Subsequently, second fermentation was continued for additional 12-24 h for aging purpose. Fermented product was then dried and powdered. Dextran sodium sulfate (DSS) with a molecular weight of 36,000-50,000 was purchased from MP Biomedicals, LLC.

Experimental procedure. The experimental design was approved by the Institutional Ethics Review Committee for animal experiments at Gifu University.

Experiment 1. The ApclMin/+ mice (5 weeks of age) were divided into 3 groups (G1, G2 and G3). The ApclMin/+ littermates (5 weeks of age) were also divided into 3 groups (G4, G5 and G6) as shown in Fig. 1. Mice in groups 1 and 4 were maintained on control diet (CE-2), groups 2 and 5 were fed with 5% FBRA in diet, and groups 3 and 6 were fed with 10% FBRA in diet. All mice were sacrificed at 20 weeks of age.

Experiment 2. As shown in Fig. 2, the ApclMin/+ mice (4 weeks of age) were divided into 3 groups (G1, G2 and G3). The ApclMin/+ littermates (4 weeks of age) were also divided into 3 groups (G4, G5 and G6). Mice in groups 1 and 4 were maintained on control diet (CE-2), groups 2 and 5 were fed with 5% FBRA, and groups 3 and 6 were fed with 10% FBRA in control diet. All mice were sacrificed at 20 weeks of age.

RNA isolation and cDNA synthesis. RNA was extracted from the colonic tissue by using TRIZol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) and an RNeasy mini kit.
(Qiagen Corp., Hilden, Germany) according to the manufacturers’ instructions. The amount and quality of RNA for each sample were assessed with the NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Purified total RNA was reverse-transcribed using the SuperScript First-Strand synthesis system for the reverse transcriptase-polymerase chain reaction (RT-PCR) kit (Invitrogen, Carlsbad, CA), according to the manufacturer’s protocol.

Real-time quantitative PCR (RT-PCR). Expression levels of iNos, Cox2, Tnf, and β-actin mRNA were measured using a Light-Cycler (Roche Applied Science) and the specific sense and antisense primers. The reaction mixture contained 10.0 μl of Takara SYBR Premix Ex Taq (Takara Bio Inc.), 1.0 μl of 10 μM of each primer, 3.0 μl of distilled water and 5.0 μl of specific cDNA. Oligonucleotide primers were: iNos, 5'-GTT CTCAGCCTCAACATAAAGA-3' and 5'-GTTGGACGG GTCAATGTCAC-3'; Cox2, 5'-GCCAGGCTGAACCTCG AAACA-3' and 5'-GTCACAGGGCCACTGATACCT-3'; Tnfa, 5'-CCCCTCAACACTCAGTCT-3' and 5'-GCT ACGAGGCTGACTCAG-3'; β-actin, 5'-CATCGTTTAAA GCCCTATGCAAC-3' and 5'-ATGGAGCCACCGAT CCACA-3'.

Immunohistochemical staining. The avidin-biotin peroxidase complex (ABC) technique was used for immunohistochemical studies. The paraffined sections were deparaffinized, rehydrated in PBS. Then, placed in 10 mmol/l citrate buffer (pH 6.0), and heated in a Pascal pressure cooker (Dako) programmed for 1 min at 120˚C for pretreatment. The endogenous peroxidase activity was blocked by incubation for 20 min in 0.3% H2O2. After washing 3 times with PBS, the sections were preincubated with a normal blocking serum for 30 min at room temperature and then incubated with primary antibodies, which are specific for Ki67 (TEC-3, 1:100 dilution, Dako), overnight at 4˚C. Subsequently, the sections were incubated with biotinylated secondary antibodies (1:250 dilution, KPL) for 30 min at room temperature, followed by incubation with avidin-coupled peroxidase (Vectastain® Elite ABC kit) for 30 min at room temperature. The sections were developed with 3,3V-diaminobenzidine (DAB), counterstained with hematoxylin and mounted coverslips.

Table I. The liver weights, kidney weights, and the length of large bowel.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Treatment</th>
<th>No.</th>
<th>Body (g)</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
<th>Length of large bowel (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control diet</td>
<td></td>
<td>25.2±3.6</td>
<td>1.4±0.31</td>
<td>0.3±0.08</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% FBRA</td>
<td>24</td>
<td>22.6±2.2</td>
<td>1.3±0.3</td>
<td>0.3±0.05</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10% FBRA</td>
<td>21</td>
<td>26.7±3.0</td>
<td>1.7±0.1</td>
<td>0.3±0.08</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control diet</td>
<td></td>
<td>27.3±6.1</td>
<td>1.2±0.34</td>
<td>0.3±0.12</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% FBRA</td>
<td>11</td>
<td>27.8±4.4</td>
<td>1.2±0.19</td>
<td>0.3±0.06</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10% FBRA</td>
<td>18</td>
<td>28.8±3.9</td>
<td>1.3±0.26</td>
<td>0.3±0.13</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Means ± SD; number of examined mice; *ND, not determined. +Significantly different from group 1 by Student’s t-test (p<0.005). +Significantly different from group 1 by Student’s t-test (p<0.01). +Significantly different from group 4 by Student’s t-test (p<0.05).
t-test. The Ki67 positive cell index was analyzed by the Welch’s t-test. Expression levels for iNos, Cox2, and Tnf mRNA were analyzed by the Mann-Whitney U test.

Results

Administration of FBRA had no effect on colonic tumorigenesis in Apc\textsuperscript{Min/+} mice. In Experiment 1, we examined the effects of FBRA administration on the development of colorectal tumors in Apc\textsuperscript{Min/+} mouse, a model for FAP. Although there were significant differences in the final body weight between groups 1 and 2 (p<0.005) and the liver weight between groups 1 and 3 (p<0.01), no evidence of toxicity was confirmed by the histological analysis as shown in Table I. All mice in groups 1, 2, and 3 developed the tumors in the colon but no tumors were found in mice in groups 4, 5, and 6. There were no differences in the incidence, multiplicity or size of tumors in colon among the groups 1, 2 and 3 as shown in Table II. Additionally, there were no difference in the Ki67 positive index in colon tumors between FBRA-treated and control diet-treated groups in either Apc\textsuperscript{Min/+} or Apc\textsuperscript{+/+} mice (data not shown).

Administration of FBRA inhibited the inflammation-related colorectal tumorigenesis. To examine the effect of FBRA on inflammation-related colorectal tumorigenesis, we fed DSS-treated Apc\textsuperscript{Min/+} mice with FBRA and examined the development of colon tumors in Experiment 2. During 8 weeks of experimental period, about one-third of total mice, which were exposed to DSS, in groups 1, 2 and 3 died because of severe colitis due to DSS treatment. However, there was no significant difference in the surviving rate among groups 1, 2 and 3. During the experimental period, there were significant differences in the body weight between groups 1 and 2 and 3 and 4 (p<0.005 and 0.001, respectively), and the liver weight between groups 1 and 3 (p<0.01). Histologically, no evidence of toxicity was found in any group, and no difference in the Ki67 positive index was observed between FBRA-treated and control diet-treated groups in either Apc\textsuperscript{Min/+} or Apc\textsuperscript{+/+} mice (data not shown).

Table II. The incidences, multiplicities and sizes of the tumors.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Treatment</th>
<th>No.</th>
<th>Incidence (%)</th>
<th>Multiplicity</th>
<th>Tumor size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>Control diet</td>
<td>21</td>
<td>19/21 (90)</td>
<td>2.9±1.8</td>
<td>18.1±20.9</td>
</tr>
<tr>
<td>2</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>5% FBRA</td>
<td>24</td>
<td>20/24 (83)</td>
<td>2.3±1.9</td>
<td>17.3±18.8</td>
</tr>
<tr>
<td>3</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>10% FBRA</td>
<td>21</td>
<td>20/21 (95)</td>
<td>2.5±1.7</td>
<td>21.6±23.9</td>
</tr>
<tr>
<td>4</td>
<td>Apc\textsuperscript{+/+}</td>
<td>Control diet</td>
<td>18</td>
<td>0/18 (0)</td>
<td>0.0±0.0</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Apc\textsuperscript{+/+}</td>
<td>5% FBRA</td>
<td>11</td>
<td>0/11 (0)</td>
<td>0.0±0.0</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Apc\textsuperscript{+/+}</td>
<td>10% FBRA</td>
<td>18</td>
<td>0/18 (0)</td>
<td>0.0±0.0</td>
<td>ND</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>Control diet + DSS</td>
<td>10</td>
<td>10/10 (100)</td>
<td>20.5±6.9</td>
<td>5.1±7.5</td>
</tr>
<tr>
<td>2</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>5% FBRA + DSS</td>
<td>7</td>
<td>7/7 (100)</td>
<td>11.3±5.6 *</td>
<td>5.1±5.9</td>
</tr>
<tr>
<td>3</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>10% FBRA + DSS</td>
<td>6</td>
<td>6/6 (100)</td>
<td>9.7±4.8 *</td>
<td>3.3±3.6</td>
</tr>
<tr>
<td>4</td>
<td>Apc\textsuperscript{+/+}</td>
<td>Control diet + DSS</td>
<td>11</td>
<td>0/11 (0)</td>
<td>0.0±0.0</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Apc\textsuperscript{+/+}</td>
<td>5% FBRA + DSS</td>
<td>11</td>
<td>0/11 (0)</td>
<td>0.0±0.0</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Apc\textsuperscript{+/+}</td>
<td>10% FBRA + DSS</td>
<td>8</td>
<td>0/8 (0)</td>
<td>0.0±0.0</td>
<td>ND</td>
</tr>
</tbody>
</table>

\*Number of examined mice; \#number of mice observed each lesion (%); \#number of total tumor per mouse (Mean ± SD). \*Means ± SD of the sizes of colonic tumor (mm\(^3\)); ND, not determined. \*Significantly different from group 1 by Student’s t-test (p<0.02). \*Significantly different from group 1 by Student’s t-test (p<0.005).

Table III. The effect of FBRA on the mRNA expression levels of Cox2, iNos, and Tnf\(\alpha\) in the colonic mucosa.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Treatment</th>
<th>No. #</th>
<th>Cox2</th>
<th>iNos</th>
<th>Tnf(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>Control diet + DSS</td>
<td>6</td>
<td>1.840±1.092</td>
<td>0.581±0.437</td>
<td>0.954±0.363</td>
</tr>
<tr>
<td>2</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>10% FBRA + DSS</td>
<td>4</td>
<td>0.384±0.365 *</td>
<td>0.032±0.046 *</td>
<td>0.520±0.324</td>
</tr>
<tr>
<td>3</td>
<td>Apc\textsuperscript{Min/+}</td>
<td>Control diet</td>
<td>4</td>
<td>0.134±0.173 *</td>
<td>3.450x10(^{-6})±1.879x10(^{-6})</td>
<td>2.833x10(^{-8})±5.179x10(^{-8})</td>
</tr>
<tr>
<td>4</td>
<td>Apc\textsuperscript{+/+}</td>
<td>10% FBRA</td>
<td>4</td>
<td>0.007±0.005 *</td>
<td>3.412x10(^{-6})±5.910x10(^{-7})</td>
<td>7.880x10(^{-9})±7.349x10(^{-9})</td>
</tr>
</tbody>
</table>

\*Means ± SD; \#number of examined mice. \*Significantly different from group 1 by Student’s t-test (p<0.04). \*Significantly different from group 1 by Student’s t-test (p<0.02). \*Significantly different from group 1 by Student’s t-test (p<0.03). \*Significantly different from group 2 by Student’s t-test (p<0.03).
and 3. Additionally, there were no differences in the final body weight, the weight of liver, and in the length of large bowel among the groups at the time of sacrifice, but there was a significant difference in the weight of kidney between group 4 and 6 (p<0.05) as shown in Table I.

In this experiment, all mice in groups 1, 2, and 3 developed tumors in the colon, but no colonic tumor was found in mice in groups 4, 5, and 6. Administration of 5% FBRA (group 2) and 10% FBRA (group 3) significantly suppressed the multiplicity of total tumors in the colon in comparison with group 1 (p<0.02 and p<0.005, respectively). However, there was no significant difference in the size of colonic tumors between FBRA-treated groups (groups 2 and 3) and control diet group (group 1) as shown in Table II.

**FBRA significantly decreased the mRNA expression levels of Cox2 and iNos.** In order to investigate the molecular mechanisms of the suppressing effect of FBRA against inflammation-related colorectal tumorigenesis in DSS-treated mice, we fed DSS-treated and DSS-untreated Apc<sup>min/+</sup> mice with 10% FBRA, and examined the mRNA expression levels of the inflammation-related genes, Cox2, iNos, and Tnfα in Experiment 3. The DSS treatment significantly increased the expression levels of Cox2, iNos, and Tnfα in colonic mucosa (p<0.03) as shown in Fig. 3. Importantly, administration of 10% FBRA significantly suppressed the mRNA expression levels of Cox2 (group 2; 0.38±0.0365, vs. group 1; 1.8±0.192, p<0.04) and iNos (group 2; 0.03±0.046, vs. group 1; 0.58±0.0437, p<0.02). In contrast, no significant difference was detected in the expression level of Tnfα (group 2; 0.52±0.324, vs. group 1; 0.95±0.363) in comparison with control diet group (Table III).

**Administration of FBRA significantly decreased the cell proliferative index in DSS-treated Apc<sup>min/+</sup> and Apc<sup>−/−</sup> mice.** To further examine the cell kinetics of colonic crypt cells in DSS-treated and DSS-untreated mice, we performed immunohistochemical analyses for the expression of Ki67, a marker for cell proliferation using the colon sections from the mice in Experiment 3. FBRA administration in DSS-treated mice significantly decreased the Ki67 positive cell index in comparison with control diet group (group 2; 0.14±0.08, vs. group 1; 0.48±0.13) but had no effect on cell proliferative index in DSS-untreated mice (group 4; 0.14±0.06, vs. group 3; 0.15±0.05) as shown in Fig. 4, suggesting that suppressive effects of FBRA on cell proliferation is closely associated with colitis.

**Discussion**

Several studies indicate that dietary factors play an important role in the pathogenesis of inflammatory bowel disease and colorectal cancer (8,9,11,12,17-19). A number of studies have shown the chemopreventive effect of fermented brown rice and rice bran (FBRA) on several types of cancer, including colorectal cancer (12-15). In contrast to previous findings that FBRA significantly suppressed azoxymethane-induced colorectal carcinogenesis in rats, in the present study, FBRA has no effect on the tumor development of the colon in Apc<sup>min/+</sup> mice, a hereditary carcinogenesis model of the colon. The results indicate that the suppressive effects of FBRA on colorectal carcinogenesis are different depending on models for colon cancer. It is noteworthy that the administration of FBRA significantly suppressed the total number of colonic tumors in DSS-treated Apc<sup>min/+</sup> mice. The results clearly indicate that FBRA specifically inhibits inflammatory-related colorectal carcinogenesis in Apc<sup>min/+</sup>-genetic background. Given the previous findings by Kataoka et al (16) in which FBRA suppresses DSS-induced inflammation in the colon, the mode of chemopreventive action of FBRA may be attributable to be the anti-inflammatory activity. Consistent with the hypo-thesis, administration of FBRA prevents the shortening of large bowel of DSS-treated Apc<sup>min/+</sup> mice, one of the biological parameter of severity of colonic inflammation.

Generally, hyperproliferation is suggested to be related to carcinogenesis of many organs (20-22). Therefore, the control of cell proliferation in the target organs is regarded as an important strategy for chemoprevention. A number of compounds consisting FBRA revealed chemopreventive actions that are accompanied by the decreased proliferative activities in the target organs. It is important to note that the administration of FBRA significantly decreased the Ki67 positive cell index in colonic crypts of DSS-treated mice, whereas such effects were not observed in colonic crypts of non-treated control mice. Thus, the suppressive effect on cellular proliferation in tumor cells might be one of the important mechanisms of the chemopreventive effects of FBRA.

Nitric oxide (NO) is involved in many of the pathophysiological processes that lead to colon cancer development and progression. The role of NO in carcinogenesis is not well defined and appears to be complex due to divergent functional activities under normal and pathophysiological conditions (23-25). It is probable that the high sustained levels of NO generated by iNos, the inducible and Ca<sup>2+</sup>-independent isozyme of NOs, can produce multiple types of damage and lead to an accumulation of gene mutations that contribute to malignant transformation (25,26). Evidence from both in vitro and in vivo
experiments support that NO and its reactive metabolite peroxynitrite stimulate Cox2 activity leading generation of tumor growth enhancing prostaglandins and influence colon tumorigenesis (27-29). Herein we showed that the expression levels of iNOS and Cox2 were strongly increased in the colonic tissues of mice treated with DSS in comparison with the untreated mice, suggesting that the increased expressions of iNOS and Cox2 are involved in the DSS-induced inflammation. It is important that the administration of FBRA significantly decreased the mRNA expression levels of both iNOS and Cox2 in the colonic tissues of mice in comparison with the control mice.

FBRA is a processed food prepared by fermenting brown rice and rice bran with Aspergillus oryzae. The nutritional and sanitary advantage of fermentation has been recognized, although the details are not well known. Fermented soy bean paste or soy sauce, which prepared by fermenting with Aspergillus oryzae, has been found to be more stable against lipid peroxidation than unfermented soybeans, because Aspergillus oryzae-fermented soybean products contain several antioxidants such as 6-hydroxydiazidei, 8-hydroxydiazidei and 8-hydroxyugenistein more abundantly than unfermented soybeans (30). These 8-hydroxysiflavones are reported to possess greater 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and antiproliferative activity than the corresponding isoflavone analogs (30,31). Furthermore, these 8-hydroxysiflavones and 6-hydroxydiazidei showed high antimutagenic activity (31,32). Several epidemiological and preclinical studies suggested the chemopreventive effects of such fermented soybean products (33-35). Dihydroferulic acid and dihydrosinapic acid, which were isolated from unpolished rice vinegar (Kurosu), were suggested as the major constituents responsible for radical scavenging activity of Kurosu (36). These acids are produced in Kurosu through the process of the fermentation from ferulic acid and sinapic acid. Rice bran contains ~20% oil which contains several bioactive polyphenols including ferulic acid, protocatechuic acid, sinapic acid, and vanillic acid (37). Thus, it is possible that FBRA has similar mechanistic aspects to the fermented soybeans or Kurosu.

In conclusion, we demonstrated that the administration of FBRA inhibits inflammation-related colorectal tumorigenesis induced by DSS in Apc(Min+) mice. Our results also suggest that a reduction of Cox2 and iNOS mRNA expression levels and a suppression of cell proliferative activity in colonic epithelium may be involved in such chemopreventive effects of FBRA.

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