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Effects of Miso and NaCl on the Development of Colonic Aberrant Crypt Foci Induced by Azoxymethane in F344 Rats

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Abstract: The present study was designed to investigate the effects of dietary miso and NaCl supplementation on the development of aberrant crypt foci (ACF) induced by azoxymethane (AOM) in male F344 rats. A total of 76 rats, six weeks of age, were divided into seven groups and given weekly subcutaneous injections of AOM (15 mg/kg body wt) for three weeks to induce ACF. The animals were placed on diets one week before the first AOM dosing. Group 1 was :d a normal diet as a control. Groups 2–4 were fed diets containing 5%, 10%, and 20% miso, respectively, and Groups 5 and 6 were fed diets containing 2.2% and 4.4% NaCl, respectively, for five weeks. Group 7 was fed a normal diet without carcinogen exposure. Dietary miso inhibited the development of ACF in a dose-dependent manner ($y = -3.9x + 140$, $r = -0.94$). Thus the mean numbers of ACF per colon were significantly lower in Groups 3 and 4 than in Group 1 ($p < 0.01$), and there were fewer aberrant crypts per colon in Group 4 than in Group 1. NaCl supplementation was associated with fewer ACF, but this was not statistically significant. 5-Bromo-2'-deoxyuridine labeling indexes in the colonic epithelium were significantly lower in Group 3 than in Group 1 ($p < 0.05$). The present results indicate that dietary miso could act as a chemopreventive agent for colon carcinogenesis.

Introduction

Colorectal cancer is the second most frequent cause of cancer mortality in the United States and the third most common cancer worldwide (1). Recently, the progressive introduction of Western dietary habits has been paralleled by an increase in colon and breast cancer. Miso is fermented from soybeans, rice, wheat, or oats, and its major constituents are vitamins, microorganisms, salts, minerals, plant proteins, carbohydrates, and fat. It has traditionally been used in the daily diet as a flavor for food in Japan and some other parts of Asia and is still one of the essential ingredients for Japanese-style cooking. We earlier reported chemopreventive effects of miso against intestinal injury in X-irradiated mice (2). It has also been reported that miso reduces the occurrence of liver and gastric tumors in experimental animals (3). Aberrant crypt (AC) foci (ACF) are putative preneoplastic lesions of colon cancer that are utilized currently as biological end points to evaluate modulation of colonic carcinogenesis. Generally, 100% miso paste includes about 2.2% NaCl, and we have reported that a 2.2% NaCl supplement promotes gastric tumorigenesis after initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rats (4). However, if the miso diet has blocking and suppressing effects against azoxymethane (AOM)-induced ACF, it may be a possible chemopreventive agent against increasing colon cancer. In this study, we have examined the chemopreventive potential of miso and NaCl supplements in the colon.

Materials and Methods

Animals

Male F344/DuCrj rats, six weeks of age at commencement, were purchased from Charles River Japan and housed five to a polycarbonate cage under constant conditions of temperature (24 ± 2°C) and relative humidity (55 ± 10%), with a 12:12-hour light-dark cycle. The animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" established by Hiroshima University. All rats were fed a commercial diet (MF, Oriental Yeast, Tokyo, Japan) (Table 1) alone or with added miso and NaCl. Miso was supplemented into biscuits at 5%, 10%, and 20% dry red miso (Miso Central Institute, Tokyo, Japan) (Table 1). The 10% miso diet corresponds to two or three bowls of Japanese-style miso soup per day. Similarly, NaCl was supplemented into biscuits at 2.2% and 4.4% NaCl (special grade, Wako Pure Chemical, Osaka, Japan). The concentration of 2.2% NaCl corresponds to the concentration of pure 10% miso paste. Normal tap water was also provided ad libitum. Constituents of miso are shown in Table 1.

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Table 1. Composition of Miso and MF Diet[a]

	Dry Red Miso	MF Diet
Water	1.8	8.6
Protein	21.0	24.0
Fat	12.1	5.1
Carbohydrate	38.6	54.0
Fiber	2.3	3.4
Ash	24.1[b]	5.9[c]
Salt	21.9	0.32
Isoflavone	0.05	ND

a: Values are expressed as percent. Dry red miso (357 kcal/100 g) was obtained from Miso Central Institute and MF diet (357 kcal/100 g) from Oriental Yeast. ND, not determined.
b: 0.08% Ca and 0.08% Mg.
c: 1.11% Ca and 0.24% Mg.

Carcinogen

AOM was purchased from Sigma Chemical (St. Louis, MO).

Experimental Procedure

A total of 76 rats were divided into seven groups. Starting at seven weeks of age, they were given weekly subcutaneous injections of AOM (15 mg/kg body wt) for three weeks to induce ACF. The rats were fed the following diets for five weeks, starting one week before the first AOM dosing. The animals were sacrificed two weeks after the last AOM injection.

Experimental Groups

The animals in Groups 1 and 6 were injected with AOM. The animals in Groups 1 and 7 were fed the normal MF diet throughout the experimental period as controls. The animals in Groups 2–4 were fed diets containing 5%, 10%, and 20% miso, respectively. The animals in Groups 5 and 6 were fed diets containing 2.2% and 4.4% NaCl, respectively.

Autopsy of Animals

One hour before the termination, 5-bromo-2′-deoxyuridine (BrdU) was injected into the peritoneal cavity (0.005 mg/kg body wt). Autopsy was performed under ether anesthesia, at which time the body and major organ weights were measured.

Visualization and Histological Examination

At the termination of the studies, the colon was removed, flushed with saline, slit open longitudinally from cecum to anus, placed on a paper towel, and fixed in 10% buffered formalin for 24 hours. Following the protocol, cited by Bird and Magnuson (5), the fixed colons were stained with 0.5% methylene blue for 15–30 minutes. The stained colons were then placed on a glass slide with the luminal side up. By viewing the stained colons with the light microscope at a magnification of ×20–30, the colons were assessed for the presence of ACF. The number of ACF per colon, the number of AC per colon, and the number of AC per focus were determined. Immunohistochemical analysis of BrdU labeling was performed for colonic mucosa from rats at random in Groups 1–6. The BrdU staining method was as follows. After deparaffinization, anti-BrdU clone Bu20a (Dakopatts) was applied to the specimens, and they were stained by Vectastain Universal quick kit (Vector Laboratories, Burlingame, CA). BrdU-positive cells in the colonic mucosal epithelium were counted under the microscope at a magnification of ×400. Additionally, longitudinal-sectional areas of the descending colonic epithelium per visual field were recorded at a magnification of ×200 using the same section used by the color image analyzer. Statistical significance was determined with Dunnett's method for multiple comparisons using logarithmic transformation and Student's *t*-test.

Results

General Observations

Diet intake and intake of drinking water were not different among groups (data not shown). The mean body and liver weights in each group are shown in Table 2. Average body weights of rats were significantly lower in Groups 3–5 than in Group 1 ($p < 0.05$–0.01), associated with diarrhea. Liver weights in AOM-treated groups were significantly increased compared with nontreated control values (Group 7).

Table 2. Mean Body and Liver Weights[a,b]

Group No.	*n*	Body Weight, g	Liver Weight, g	Relative Body Weight, g/100 g body wt
1 (AOM)	17	245.7 ± 12.4	8.77 ± 1.03	3.56 ± 0.38
2 (AOM + 5% miso)	10	241.4 ± 6.7	8.58 ± 0.21	3.55 ± 0.05
3 (AOM + 10% miso)	10	233.8 ± 11.0*	8.62 ± 0.88	3.70 ± 0.23
4 (AOM + 20% miso)	10	213.3 ± 6.8†	7.91 ± 0.43	3.71 ± 0.09
5 (AOM + 2.2% NaCl)	10	232.9 ± 6.9†	8.24 ± 0.29	3.50 ± 0.15
6 (AOM + 4.4% NaCl)	10	237.1 ± 8.3	8.58 ± 0.42	3.63 ± 0.06
7 (Nontreated)	9	266.3 ± 12.1	8.16 ± 0.23	3.23 ± 0.10

a: Values are means ± SD. AOM, azoxymethane.
b: Statistical significance is as follows: significantly different from Group 1: *, $p < 0.05$; †, $p < 0.01$.

Table 3. Effects of Dietary Miso and NaCl on AOM-Induced ACF in Rat Colons[a,b]

Group No.	Incidence	ACF/Colon	AC/Colon	AC/Focus
1 (AOM)	17/17	137.88 ± 43.98	253.70 ± 73.80	1.66 ± 0.77
2 (AOM + 5% miso)	10/10	134.10 ± 52.20	240.90 ± 92.90	1.83 ± 0.88
3 (AOM + 10% miso)	10/10	85.60 ± 70.50*	163.00 ± 130.80	1.94 ± 0.96
4 (AOM + 20% miso)	10/10	66.30 ± 62.90*	124.40 ± 77.90*	1.88 ± 0.91
5 (AOM + 2.2% NaCl)	10/10	115.90 ± 65.20	199.50 ± 92.60	1.82 ± 0.85
6 (AOM + 4.4% NaCl)	10/10	100.40 ± 62.90	159.80 ± 95.60	1.61 ± 0.79
7 (Nontreated)	0/9	0	0	0

a: Values are means ± SD. ACF, aberrant crypt foci; AC, aberrant crypts.
b: Statistical significance is as follows: *, significantly different ($p < 0.01$) by using Dunnett's method of multiple comparison.

Liver and relative liver weights did not differ significantly among the AOM-treated groups.

Colonic ACF

Table 3 summarizes data for the mean number of ACF per colon, total number of AC per colon, and mean number of AC per focus. The rats treated with AOM (Groups 1–6) showed a 100% incidence of ACF. The average number of ACF per colon was significantly decreased by feeding 10% miso (Group 3) and 20% miso (Group 4) compared with Group 1 ($p < 0.01$). AC per colon were significantly decreased by feeding the 20% miso diet (Group 4) compared with Group 1 ($p < 0.01$). NaCl-supplemented groups showed fewer ACF, but this was not statistically significant. There were no significant differences in number of AC per focus among the groups.

BrdU Labeling Indexes

BrdU labeling indexes of the colonic mucosal epithelium were significantly lower in Group 3 than in Group 1 ($p < 0.05$) (Table 4). Height of the colonic mucosal epithelium was not significantly changed in any group (data not shown), but the longitudinal-sectional areas of the descending colon epithelium were significantly smaller in Group 5 (88,177 ± 12,741 μm^2) than in Group 1 (101,354 ± 16,201 μm^2, $p < 0.05$).

Discussion

ACF are putative preneoplastic lesions of colon cancer that are currently utilized as a biological end point to evaluate colon carcinogenesis and its modulation (5). In the present study, dietary administration of miso inhibited the development of AOM-induced ACF in a dose-dependent manner in the rat colon ($y = -3.9x + 140$, $r = -0.94$). Specifically, the average number of ACF per colon in animals supplemented with 10% or 20% miso and the AC per colon in the 20% miso group were significantly decreased ($p < 0.01$). NaCl-supplemented groups also showed a trend toward a lower incidence of ACF and AC per colon, but this was not statistically significant. Ito and co-workers (6) earlier reported that miso reduced spontaneous or fission neutron-induced liver tumors in mice. Watanabe and colleagues (7) found that miso reduced *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced gastric tumors in rats (7). Recently, Gotoh and associates (8,9) described an inhibitory effect of miso on the occurrence of rat mammary carcinogenesis induced by *N*-nitroso-*N*-methylurea. Thus miso supplementation may exert inhibitory effects against various cancers.

Colorectal and mammary cancers were uncommon in Japan in the past, but recently there has been an increase because of the progressive introduction of Western dietary habits. Hirayama (10) reported that frequent miso soup consumption is strongly associated with a reduction in gastric cancer mortality, and Watanabe and colleagues (7) provided supportive experimental evidence. Asahara and others (11) found that the induction of mutations by Trp-2 was blocked by miso when an Ames assay was performed. Herman and co-workers (12) found an inhibitory effect of soy foods, i.e., miso, on spontaneous and chemically induced mammary carcinogenesis. Soy foods contain significant amounts of two isoflavones, genistein and daidzein, which have various biological activities and antitumorigenic effects. These isoflavones, especially genistein, have been proposed to inhibit tyrosine-specific protein kinases (13), DNA topoisomerases I and II (14), angiogenesis (15), and the growth of cultured human gastric cancer cell lines (16) and to cause cell cycle arrest at G_2-M (17). Recently, Fukutake and colleagues (18) reported that genistein is present at higher levels in miso than in other soybean-related products, such as soy powder, soy milk, tofu, natto, and soy sauce. Genistein has been proposed as the most effective constituent to inhibit mammary cancer, also possessing antiestrogenic activity (9,19–21). Gotoh and associates (8) reported that administration of biochanin A, a genistein precursor, inhibits the

Table 4. BrdU Labeling Indexes in Colonic Epithelium[a,b]

Group No.	*n*	Labeling Index
1 (AOM)	40	0.55 ± 0.17
2 (AOM + 5% miso)	17	0.53 ± 0.15
3 (AOM + 10% miso)	7	0.39 ± 0.11*
4 (AOM + 20% miso)	13	0.50 ± 0.15
5 (AOM + 2.2% NaCl)	10	0.48 ± 0.17
6 (AOM + 4.4% NaCl)	14	0.47 ± 0.14

a: Values are means ± SD; *n*, number of samples. BrdU, 5-bromo-2'-deoxyuridine.
b: Statistical significance is as follows: *, significantly different from Group 1 ($p < 0.05$).

development of rat mammary tumors. However, we previously found that its administration did not reduce colonic ACF in rats induced by AOM (22). Thus inhibitory effects of a miso diet against colorectal and mammary cancer may be due to different constituents. Kawamori and others (23) reported that a saponin in soybean inhibited ACF induction by AOM in rats. Calcium has been hypothesized as being a regulator of cell proliferation in the colon, and dietary intake of calcium inhibits experimental carcinogenesis (24). It is considered that the inhibitory effect of miso against ACF induced by AOM is not due to genistein in soybean NaCl, but rather to minerals or other constituents that are generated in the process of fermentation.

In the present study, the BrdU labeling index of the 10% miso-supplemented group was significantly lower than that of the control group. In addition, the longitudinal-sectional areas of descending colon were significantly smaller in Group 5 (2.2% NaCl) than in Group 1. However, BrdU labeling indexes and longitudinal-sectional area did not directly correlate with the results of ACF and AC induction. Thus it was suggested that the development of ACF is inhibited by miso but is not dependent on depressed cell proliferation in the colonic epithelium.

In conclusion, the results of the present study indicate that dietary miso supplementation is useful for colonic cancer prevention. Further experiments are required to evaluate the effects of individual components, including minerals, in miso.

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References

1. Tajima, K, Hirose, K, Nakagawa, N, Kuriishi, T, and Tominaga, S: Urban-rural difference in the trend of colorectal cancer mortality with special reference to the subsites of colon cancer in Japan. *Jpn J Cancer Res* 76, 717–728, 1985.
2. Watanabe, H, Takahashi, T, and Ishimoto, T: The effect of miso diet on small intestinal damage in mice irradiated by X-ray. *Miso Sci Technol* 39, 29–32, 1991. [In Japanese]
3. Watanabe, H, Masaoka, Y, Gotoh, T, Fujimoto, N, and Ito, A: Effects of miso in reducing risk of liver and gastric tumors in experimental animals. In *Food Factors for Cancer Prevention*, H Ohigashi, T Osawa, J Terao, S Watanabe, and T Yoshikawa (eds). Tokyo: Springer-Verlag, 1997, pp 351–354.
4. Watanabe, H, Tanizaki, M, Masaoka, Y, Syouji, S, Ito, A, et al.: Inhibition of gastric tumors by administration with miso diet during *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) treatment in rats. *Miso Sci Technol* 45, 107–115, 1997. [In Japanese]
5. Bird, RP, and Magnuson, BA: Ability of aberrant crypt foci characteristics to predict colonic tumor incidence in rats fed cholic acid. *Cancer Res* 53, 4499–4504, 1993.
6. Ito, A, Watanabe, H, and Basaran, N: Effects of soy products in reducing risk of spontaneous and neutron-induced liver tumor in mice. *Int J Oncol* 2, 773–776, 1993.
7. Watanabe, H, Tanizaki, M, Ando, Y, Yamada, K, Gotoh, T, et al.: Effect of miso diet on gastric tumors induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in rats. *Miso Sci Technol* 43, 214–218, 1995.
8. Gotoh, T, Yamada, K, Yin, H, Ito, A, Dohi, K, et al.: Chemoprevention of *N*-nitroso-*N*-methylurea-induced rat mammary carcinogenesis by soy foods or biochanin A. *Jpn J Cancer Res* 89, 137–142, 1998.
9. Gotoh, T, Yamada, K, Ito, A, Yin, H, Kataoka, T, et al.: Chemoprevention of *N*-nitroso-*N*-methylurea rat mammary cancer by miso and tamoxifen, alone and in combination. *Jpn J Cancer Res* 89, 487–495, 1998.
10. Hirayama, T: Relationship of soybean paste soup intake to gastric cancer risk. *Nutr Cancer* 3, 223–233, 1982.
11. Asahara, N, Zhang, XB, and Ohta, Y: Anti-mutagenic and mutagen-binding activation of mutagenic pyrolyzate by microorganisms isolated from Japanese miso. *J Sci Food Agric* 58, 395–401, 1992.
12. Herman, C, Adlercreutz, T, Goldin, BR, Gorbach, SL, Hockerstedt, KAV, et al.: Soybean phytoestrogen intake and cancer risk. *J Nutr* 125, 757–770, 1995.
13. Akiyama, T, Ishida, J, Nakagawa, S, Ogawara, H, Watanabe, S, et Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 262, 5592–5595, 1987.
14. Okura, A, Arakawa, H, Oka, H, Yoshinari, T, and Monden, Y: Effect of genistein on topoisomerase activity and the growth of [Val^{12}]H-*ras*-transformed NIH3T3 cells. *Biochem Biophys Res Commun* 157, 183–189, 1988.
15. Fotsis, T, Pepper, M, Adlercreutz, H, Hase, TA, Montesano, R, et al.: Genistein, a dietary ingested isoflavone, inhibits cell proliferation and *in vitro* angiogenesis. *J Nutr* 125, 790–797, 1995.
16. Yanagihara, K, Ito, A, Toge, T, and Numoto, M: Anti-proliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res* 53, 5815–5821, 1993.
17. Matsukawa, Y, Marui, N, Sakai, T, Satomi, Y, Yoshida, Y, et al.: Genistein arrests cell cycle progression at G_2-M. *Cancer Res* 53, 1328–1331, 1993.
18. Fukutake, M, Takahashi, M, Ishida, K, Kawamura, H, Sugimura, T, et al.: Quantification of genistein and genistin in soybeans and soybean products. *Food Chem Toxicol* 34, 457–461, 1996.
19. Shutt, DA, and Cox, RI: Steroid and phyto-estrogen binding to sheep uterine receptors *in vitro*. *J Endocrinol* 52, 299–310, 1972.
20. Martin, PM, Horwitz, KB, Ryan, DS, and McGuire, WL: Phytoestrogen interaction with estrogen receptors in human breast cancer. *J docrinology* 103, 1860–1867, 1978.
21. Folman, Y, and Pope, GS: The interaction in the immature mouse of potent oestrogens with coumestrol, genistein and other utero-vaginotrophic compounds of low potency. *J Endocrinol* 34, 215–225, 1966.
22. Masaoka, Y, Watanabe, H, Tanizaki, M, Ando, Y, and Ito, A: Effect of a miso diet on colonic aberrant crypt foci to azoxymethane. In *Recent Advances in Gastroenterological Carcinogenesis. I*, E Tahara, K Sugimachi, and T Oohara (eds). Bologna: Monduzzi-Editore, 1996, pp 1181–1185.
23. Kawamori, T, Tanaka, T, Hara, A, Yamahara, J, and Mori, H: Modifiyng effects of naturally occurring products on the development of colonic aberrant crypt foci induced by azoxymethane in F344 rats. *Cancer Res* 55, 1277–1282, 1995.
24. Appleton, GVN, Davies, PW, Bristol, JB, and Williamson, RCN: Inhibition of intestinal carcinogenesis by dietary supplementation with calcium. *Br J Surg* 74, 523–525, 1987.