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Inhibition by long-term fermented miso of induction of gastric tumors by N-methyl-N'-nitro-N-nitrosoguanidine in CD (SD) rats

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Abstract. The present study was designed to investigate the effects of fermented miso in the diet on the induction of gastric tumors by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in male CD (SD) rats: A total of 120 animals, 6 weeks of age, were divided into 6 groups and given MNNG (100 ppm) in the drinking water for 16 weeks. Starting 1 week before the carcinogen treatment the rats were fed a normal control MF solid diet, or the same diet containing 10% long-term fermented, medium- or short-term fermented miso, or 1% NaCl until the end of the MNNG exposure period. They were then maintained on the MF control diet and normal tap water until the autopsy time point at 52 weeks. The long-term fermented miso significantly reduced the size of the gastric tumors as compared with the other groups. The present results thus indicate that dietary supplementation with long-term fermented miso could act as a chemopreventive agent for gastric carcinogenesis.

Introduction

Miso is produced by fermentation of soybeans, rice, wheat or oats and its major constituents are vitamins, enzymes, micro-organisms, 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 required for Japanese-style cooking. Recently, there has been an increasing demand for so-called health foods, with primary prevention of cancer as one of the expected effects. Epidemiological studies in Japan by Hirayama (1) indicated that this fermented soybean product might have an inhibitory effect on gastric cancer. It has also been found to reduce the risk of liver tumors occurring spontaneously (2) or induced by neutron irradiation alone or in combination with diethylnitrosamine (DEN) (3). Furthermore, inhibition has been reported for N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induced gastric tumorigenesis (4,5), development of azoxymethane-induced aberrant crypt foci (ACF) (6), and N-nitroso-N-methylurea (MNU)-induced rat mammary carcinogenesis (7-9). Recently, our experimental studies provided evidence that long-term fermented miso is quite effective in aiding recovery of stem cells in the small intestinal crypt after irradiation damage (10), also decreasing development of azoxylmethane-induced ACF (11). To determine whether soybeans themselves or the fermentation process may be important, the present study was conducted to assess the effects of miso at various fermentation-stages on the 'initiation' phase of MNNG-induction of rat glandular stomach tumors.

Materials and methods

Animals. One hundred and twenty, six-week-old male Crj: CD (SD) rats (Charles River Japan Inc. Hino) were used in the present study. They were housed 3 or 4 to a polycarbonate cage and kept under constant conditions of temperature (24±2˚C) and relative humidity (55±10%) with a 12-h light/12-h dark cycle. The animals were maintained under the guidelines set forth in the 'Guidelines for the Care and Use of Laboratory Animals' established by Hiroshima University.

MNNG (N-methyl-N'-nitro-N-nitrosoguanidine). The carcinogen was purchased from Aldrich Chemical Co. Inc. Milwaukee, WI and dissolved in distilled water at a concentration of 100 mg/l just before use. This solution was given to rats *ad libitum* for 16 weeks from light-opaque bottles exchanged at 2 or 3-day intervals.

Diet. All rats were fed a commercial diet (MF; Oriental Yeast Co., Tokyo, Japan) alone or with added miso or NaCl. Dry red miso after short- (3-4 days), medium- (4 months) and long-term fermentation (6 months) was supplemented into solid biscuits at 10% (Miso Central Institute, Tokyo, Japan). Similarly, NaCl was added at 1% (special grade, Wako Pure Chemical, Osaka, Japan), this concentration corresponding to the concentration of salt achieved with the 10% miso paste was also provided *ad libitum*. The diets were supplied with normal tap water *ad libitum* for 1 week previously and throughout the period of MNNG exposure, and then the MF control diet and normal tap water were provided until the

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Table I. Intake of diet and MNNG.

Group	Diet (g/day/animal)	MNNG (water, ml/day/animal)	Diet + water
MF	15.0±0.9	17.6±1.4	32.6
Short-term	14.0±0.7	19.4±1.8*	33.4
Medium-term	13.1±1.2**	18.5±2.4	31.6
Long-term	14.1±0.7	19.7±1.7**	33.8
NaCl	13.9±0.8	18.8±1.9	32.7
Control	23.1±1.2	30.2±12.0	53.3

Control (without MNNG) values are significantly different from other groups (P<0.01). *Significantly different from the MF value (P<0.05). **Significantly different from the MF value (P<0.01).

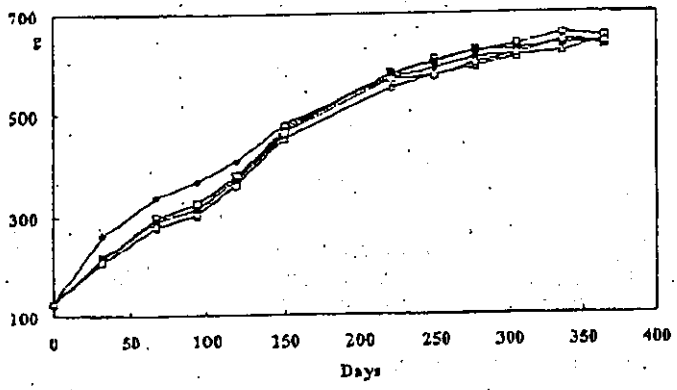

Figure 1. Body weight curve: •, MF; ○, early; △, middle; ▪, long; ○, NaCl.

autopsy time point at 52 weeks. Diet and drinking water consumption was measured at the beginning and end of MNNG treatment.

Pathology. Animals were sacrificed and autopsied if they became moribund and all remaining rats were sacrificed 12 months after the commencement of MNNG treatment. The stomachs were removed, opened along the greater curvature and extended on cardboard for inspection. The small and large intestines were similarly processed. The location of individual tumors in the small intestine was recorded by measuring the distance from the pyloric ring. Number and size of individual tumors in the large intestine were recorded and their locations were noted by measuring the distance from the anus. All tissues were fixed in 10% neutral formalin. Tumors in the stomach were classified into two types: adenomas and adenocarcinomas, the latter being either well differentiated (100% well differentiated adenocarcinoma cells in the tumor) or poorly differentiated (over 70% of the tumor consisting of poorly differentiated adenocarcinoma cells), invading the muscularis mucosa or further.

Statistical significance. Statistical significance was determined with the Dunnett method for multiple comparisons, χ^2 and Student's t-test.

Results

Intake of diet in medium-term miso group was significantly decreased as compared with the MF value. MNNG intake i the controls was significantly lower than in the short-term fermented (P<0.05) and long-term fermented (P<0.01) groups (Table I). Intake of diet plus MNNG was 31.6 to 33.8 without any significant variation.

Body weight curves are shown in Fig. 1. One month after the commencement of MNNG treatment the body weights in the MF group were significantly greater than in the other groups, this continuing until day 94 (P<0.01), and for the medium-term fermented, long-term fermented and NaCl groups until day 119 (P<0.05). There were no significant inter-group differences in body weights from 150 days until the end of the experiment.

Since small intestinal tumors appeared at 154 days after the first MNNG treatment, rats which survived beyond this point were counted in the effective numbers. Mean survival, body, absolute and relative organ weights did not significantly vary among the groups (Tables II and III).

Table IV summarizes data for tumor induction. Incidences of total and single site tumors, as well as cumulative incidences did not significantly differ among the groups. However average size of gastric tumors in the long-term fermented group was significantly smaller than the MF value (P<0.05) and this also extended to gastric adenocarcinomas (P<0.05). Values for the early-stage fermented miso group were significantly higher than for the medium-term fermented, MF (P<0.05) and long-term fermented cases (P<0.01). Sizes of gastric tumor per ml MNNG intake are shown in Table V.

Table II. Body and organ weights (g).

Group	Mean survival	Body	Liver	Kidney	Testis	Adrenal	Spleen	Heart
MF	348±38	614±114	17.9±4.0	3.05±0.35	3.56±0.28	0.073±0.036	1.00±0.32	1.80±0.20
Short-term	321±55	612±97	20.0±3.4	3.23±0.40	3.53±0.26	0.081±0.030	1.57±1.54	1.83±0.42
Medium-term	348±46	631±83	18.7±3.2	3.07±0.39	3.50±0.27	0.081±0.045	1.18±0.29	1.73±0.18
Long-term	343±46	621±112	20.1±4.0	3.31±0.53	3.56±0.37	0.067±0.021	1.26±0.69	1.76±0.27
NaCl	347±35	631±83	18.4±1.4	3.12±0.40	3.56±0.33	0.067±0.047	1.25±0.72	1.69±0.15
Control	369±3	715±72	21.6±3.7	3.42±0.41	3.44±0.17	0.064±0.011	0.89±0.27	1.69±0.33

Table III. Relative organ weights.

Group	Liver	Kidney	Testis	Adrenal	Spleen	Heart
MF	27.3±2.2	4.76±0.55	5.59±0.75	0.117±0.061	1.55±0.39	2.81±0.35
Short-term	29.1±2.6	4.75±0.69	5.19±0.68	0.118±0.049	2.20±1.96	2.64±0.38
Medium-term	29.1±2.9	4.81±0.39	5.54±0.76	0.129±0.071	1.86±0.51	2.73±0.35
Long-term	30.5±7.4	4.98±0.72	5.37±0.57	0.101±0.030	1.96±1.25	2.65±0.40
NaCl	28.2±2.2	4.80±0.59	5.48±0.65	0.102±0.067	1.92±1.11	2.60±0.21
Control	30.3±5.2	4.85±0.89	4.89±1.07	0.091±0.009	1.27±0.46	2.49±0.48

Table IV. Tumor incidence data.

Group	Effective no.	Incidence	No. of tumor/rat	Gastric tumor			Duodunum	Other
				ATP	Ad-Ca	Total		
MF	20	18 (90.0)	1.55±0.60	3 (15.0)	11 (55.0)	14 (70.0)	11 (55.0)	6 (30.0)
Early-term	19	16 (84.2)	1.10±0.74	2 (10.5)	6 (31.6)	8 (42.1)	8 (42.1)	5 (26.3)
Medium-term	19	17 (89.5)	1.29±0.69	8 (42.1)	6 (31.6)	14 (73.7)	6 (31.6)	3 (15.8)
Long-term	19	16 (84.2)	1.15±0.67	1 (5.3)	9 (47.4)	10 (52.6)	7 (36.8)	4 (21.1)
NaCl	19	12 (78.9)	1.00±0.58	3 (15.9)	8 (42.0)	11 (57.9)	5 (26.3)	1 (5.2)

Table V. Sizes of gastric tumor (mm).

Group	Gastric tumor[a]	Adenocarcinoma no.[b]	Average size
MF	5.17±4.45 (0.29)	11	8.0±2.9 (0.45)
Early-term	3.62±6.79 (0.19)	6	11.5±7.6 (0.59)
Medium-term	2.42±2.68 (0.13)	6	4.3±3.6[d] (0.23)
Long-term	1.78±3.34[c] (0.09)	9	2.9±4.6[c,e] (0.15)
NaCl	3.23±4.08 (0.16)	8	5.1±4.1[d] (0.27)

[a]Including negative animals. [b]Positive animal values. [c]Significantly different from the MF value ($P<0.05$). [d]Significantly different from the early-term value ($P<0.05$). [e]Significantly different from the early-term value ($P<0.01$). Numbers in brackets indicate size/MNNG intake/day.

Discussion

In the present experiments, while tumor incidences were not significantly different among the groups, gastric tumor size in the animals treated with long-term fermented miso was significantly decreased. Ito and colleagues have previously reported that the miso reduces spontaneous or fission neutron- or DEN and neutron-induced liver tumors in mice (2,3), as well as azoxymethane-induced colon aberrant crypt foci in rats (6). Effects on MNU-induced mammary tumors in rats (7-9) have also been reported. Soy foods contain significant amounts of the isoflavone, genistein, which has various biological activities and antitumorigenic effects (10,11), as well as antiestrogenic activity (12,13). Recently we found that prolonged fermentation might be very important for protection against radiation effects, being associated with prolongation of animal survival and decrease in toxicity to small intestinal crypts (14) and for decreased of ACF (15). However, to our knowledge there have been no reports regarding the effective substances in miso of different fermented stages. Further study is now needed for their elucidation.

In conclusion, the results of the present study indicate that fermented miso supplementation of the diet might be useful for gastric cancer prevention. Further experiments are required to evaluate the effects of individual components, including minerals, in miso.

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