ONCOLOGY REPORTS 6: 989-993, 1999

参考2

Influence of concomitant Miso or NaCl treatment on induction of gastric tumors by N-methyl-N'-nitro-N-nitrosoguanidine in rats

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Received March 16, 1999; Accepted May 11, 1999

Abstract. Six-week old male Sprague-Dawley (CD) rats were treated with 100 ppm N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for 16 weeks in their drinking water with control, Miso or sodium chloride (NaCl) supplemented diets. All animals were autopsied 12 months after the beginning of the MNNG treatment. Despite higher intake of MNNG in the high dose Miso and NaCl groups, the total tumor incidences were decreased compared to middle and lowest values. The glandular stomach adenocarcinoma incidences in the 10% and 5% Miso groups were significantly decreased as compared to those in the 2.2% or 1.1% NaCl groups, with the same concentration of NaCl.

Introduction

Miso is fermented from soy beans, rice, wheat or oats and its major constituents are vitamins, enzymes, 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 required for Japanese-style cooking. Recently, there has been an increasing demand for so-called health foods, with the primary prevention of cancer as one of their expected effects. Epidemiological studies in Japan by Hirayama (1) indicated that this fermented soybean product might have an inhibitory effect on gastric cancer. However, previously there has been almost no scientific evaluation of the biological effects of Miso on human health. Our studies experimentally have shown that Miso is quite effective at aiding recovery of stem cells in the small intestinal crypts after irradiation damage (2). It reduces the risk of liver tumors occurring spontaneously (3) or induced by neutron irradiation alone or in combination with diethylnitrosamine (DEN) (4). It was also found to inhibit N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induced gastric tumorigenesis in rats (5), the development of azoxymethane-induced aberrant crypt foci (ACF) (6), and N-nitroso-N-methylurea (MNU)-induced rat mammary carcinogenesis (7-9). In the present study, we investigated the effects of Miso as compared to sodium chloride (NaCl), one of its main constituents, on the initiation phase of MNNG-induction of rat glandular stomach tumors.

Materials and methods

Animals. One hundred and ninety-eight, six-week old male Crj: CD (SD) rats (Charles River Japan Inc. Hino) were used in the present study. They were housed three or four 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). MNNG was purchased from Aldrich Chemical Co. Inc. Milwaukee, WI and dissolved in distilled water at a concentration of 100 mg/liter just before use. This solution was given to rats *ad libitum* for 16 weeks from light-opaque bottles exchanged at 3 or 4 day intervals.

Diet. Miso diets were made into biscuits by combining 20%, 10% and 5% dry Miso provided by Miso Central Institute (Tokyo, Japan) with regular-MF diet (Table I). Diets supplemented with 4.4%, 2.2% and 1.1% NaCl, the equivalent amounts of the salt alone, were also produced (Oriental Yeast Co., Tokyo). The diets were supplied *ad libitum* during the initiation of MNNG, and then the MF control diet and normal tap water were provided until the autopsy time point at 52 weeks. Diet and drinking water consumption was measured at the beginning and end of MNNG treatment.

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Key words: N-methyl-N'-nitro-N-nitrosoguanidine, Miso, rat gastric tumors, biological sterilization

Table I. Composition of Miso (from Miso Central Institute) and MF diet (from Oriental Yeast Co.).

Composition	Dry red Miso	MF
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%	5.9%
Salt	21.9%	0.32%
Isoflavone	0.05%	Not determined
Calorie content	357 kcal/100 g	357 kcal/100 g

Pathology. Animals were sacrificed and autopsied when 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. The number and size of individual tumors within it 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 Dunnet method for multiple comparison, Dunnet method, χ^2 and the Student's t-test.

Results

Intake of drinking water and diet. There was no significant variation within each treatment group regarding intakes of drinking water and diets at the beginning and end of the MNNG treatment period so data were combined as summarized in Table II.

MNNG intake was increased as a result of increased consumption of water with high concentrations of Miso or NaCl in the diet, whereas diet intake itself tended to be decreased. In the MNNG treatment groups, amounts of diet plus drinking water did not vary greatly (34.1-40.2). In the diet groups without MNNG treatment, intake of diet was approximately the same, but intake of drinking water markedly increased with the Miso and NaCl concentration.

Body weight. Body weight data are shown in Table III. During the MNNG treatment, body weights were decreased as compared to the non-treated animals, and did not fully recovery thereafter (data not shown).

Table II. Diet and drinking water intake.

Group	Diet (g/day/rat)	Drinking water (ml/day/rat)	Diet + drinking water
MNNG + 20% Miso	13.2±1.6[a]	24.9±3.2[a]	38.1
MNNG + 10% Miso	15.5±2.7	21.2±2.2[a]	36.7
MNNG + 5% Miso	17.1±2.6	19.5±2.2	36.6
MNNG + 4.4% NaCl	14.3±2.4	23.1±2.4[a]	37.4
MNNG + 2.2% NaCl	19.2±2.8[b]	21.0±2.4	40.2[b]
MNNG + 1.1% NaCl	17.6±3.3	19.9±1.8	37.5
MNNG + MF	16.0±2.5	18.1±1.8	34.1
20% Miso	22.9±2.9	74.9±20.5	97.8
10% Miso	21.6±1.6	38.2±11.5	59.8
5% Miso	21.6±2.2	34.2±10.1	55.8
4.4% NaCl	22.1±1.8	47.2±10.9	69.3
2.2% NaCl	23.9±2.2	38.4±11.2	62.3
1.1% NaCl	23.8±1.4	35.1±13.7	58.9
MF	23.1±1.2	30.2±12.0	53.3

[a]Significantly different from the MNNG groups ($p<0.01$); [b]Significantly different from the MNNG groups using the Dunnet method ($p<0.05$).

Table III. Mean survival and body weights.

Group	No.	Mean survival	Body (g)
MNNG + 20% Miso	19	346±36	660±112
MNNG + 10% Miso	20	326±47	642±67[a]
MNNG + 5% Miso	19	334±60	648±114
MNNG + 4.4% NaCl	20	343±32	608±107[a]
MNNG + 2.2% NaCl	19	338±46	632±108
MNNG + 1.1% NaCl	20	362±5	642±64[a]
MNNG + MF	19	356±19	664±93
20% Miso	13	358±20	674±83
10% Miso	7	366	702±57
5% Miso	8	366±3	640±71
4.4% NaCl	6	344±54	696±18
2.2% NaCl	8	369±3	700±104
1.1% NaCl	8	368±2	669±61
MF	8	369±3	715±72

[a]Significantly different from the MNNG groups ($p<0.05$).

Induction of tumors. Since liver tumors appeared at 212 days after the first MNNG treatment, rats which survived beyond this point were counted in the effective numbers. One rat each in the MNNG + 20% Miso, MNNG + 5% Miso, MNNG +

Table IV. Tumor incidence, number and size.

Group	Total incidence (%)	Gastric tumor: Incidence (%)	Gastric tumor: Average size (mm)	Gastric tumor: Number	Small intestinal tumor: Incidence (%)	Small intestinal tumor: Average size (mm)	Small intestinal tumor: Number	Other
MNNG + 20% Miso	14/19 (73.7) (1.00±0.75)[a]	9/19 (47)	3.0±4.5	0.6±0.8	7/19 (37)	5.8±10.6	0.4±0.6	Sarcoma
MNNG + 10% Miso	17/20 (85.0) (1.15±0.75)[a]	9/20 (45)	2.3±3.5	0.6±0.7	9/20 (45)[b]	8.2±13.9	0.6±1.0	Sarcoma
MNNG + 5% Miso	10/19 (52.6) (0.63±0.68)[a]	7/19 (37)	2.0±2.9	0.5±0.7	2/19 (11)	5.0±17.5	0.2±0.5	Lymphoma Liver tumor
MNNG + 4.4% NaCl	13/20 (65.0) (0.85±0.81)[a]	8/20 (40)	2.5±3.4	0.5±0.5	8/20 (40)[b]	5.6±10.7	0.6±0.8	Lymphoma
MNNG + 2.2% NaCl	17/19 (89.5)[b] (1.32±0.67)[a]	13/19 (68)[a]	4.1±5.1	0.8±0.8	11/19 (58)[c]	11.4±17.1	0.6±0.8	Squamous cell carcinoma Plasmacytoma
MNNG + 1.1% NaCl	15/20 (75.0) (0.9±0.64)[a]	12/20 (60)	4.5±4.4[b]	0.7±0.7	6/20 (30)	4.1±9.1	0.3±0.6	Sarcoma
MNNG + MF	10/19 (53.0) (0.65±0.67)[a]	6/19 (32)	1.2±2.3	0.7±1.1	3/19 (16)	7.6±20.6	0.2±0.4	Plasmacytoma Sarcoma

[a]Number of tumors per rat; [b]Significantly different from the MNNG + MF value ($p<0.05$); [c]Significantly different from the MNNG + MF value ($p<0.01$).

Table V. Tumor multiplicity.

Group	0[a]	1[a]	2[a]	3[a]
MNNG + 20% Miso	5 (26)	9 (47)	5 (26)	0
MNNG + 10% Miso	3 (15)	12 (60)	4 (20)	1 (5)
MNNG + 5% Miso	9 (47)	8 (42)	2 (11)	0
MNNG + 4.4% NaCl	8 (40)	7 (35)	5 (25)	0
MNNG + 2.2% NaCl	2 (11)	9 (47)	8 (42)[b]	0
MNNG + 1.1%NaCl	5 (20)	12 (60)	3 (15)	0
MNNG + MF	9 (47)	9 (47)	2 (11)	0

[a]0, no tumor; 1, one organ; 2, two different organs; 3, three different organs; [b]Significantly different from the MNNG + MF value ($p<0.05$).

Table VI. Incidence of gastric lesions.

Group	No	Atypical hyperplasia	Adenocarcinoma	Total
MNNG + 20% Miso	19	3 (16)	6 (32)	9 (47)
MNNG + 10% Miso	20	4 (20)	5 (25)[a]	9 (45)
MNNG + 5% Miso	19	3 (16)	4 (21)[a]	7 (37)
MNNG + 4.4% NaCl	20	1 (5)	7 (35)	8 (40)
MNNG + 2.2% NaCl	19	4 (21)	9 (47)	13 (68)[a]
MNNG + 1.1% NaCl	20	0	12 (60)	12 (60)
MNNG + MF	19	1 (5)	5 (26)	6 (32)

[a]Significantly different from the MNNG + 1.1% NaCl value ($p<0.05$).

2.2% NaCl and MNNG + MF groups died before day 212. There were no tumors in animals not receiving MNNG. Arteritis appeared in animals receiving 4.4% NaCl.

Total tumor incidences for the MNNG + 10% Miso (85.0%), MNNG + 2.2% NaCl (89.5%) and MNNG + 1.1% NaCl (75.0%) treatment groups were significantly increased as compared with the MNNG + MF diet values (53.0%). The numbers of tumors per rat for the MNNG + 10% Miso and MNNG + 2.2 % NaCl treated groups were increased (Table IV). Multiple tumors were significantly more frequent in the MNNG + 2.2% NaCl treated animals than in MNNG + MF treated rats (Table V). Incidences of total gastric tumors were only significantly altered by 2.2% (Table VI). Tumor sizes for the MNNG + 1.1% NaCl treated groups were also significantly increased. All gastric tumors were of well-differentiated type. The incidences of adenocarcinomas in the MNNG + 10% and 5% Miso groups were significantly decreased as compared to the MNNG+2.2% NaCl and MNNG + 1.1% NaCl cases.

Incidences of small intestinal tumors in the MNNG + 10% Miso and MNNG + 4.4% and 2.2% NaCl were significantly increased. Sizes of small intestinal tumors in the MNNG + 4.4% NaCl and MNNG + 2.2% NaCl groups were significantly increased.

Other tumors were sarcomas, lymphomas, plasmacytoma, liver tumor and squamous cells carcinoma. without any significant intra-group variation.

Discussion

In the present experiments, MNNG intake was increased by high concentrations of Miso or NaCl. The total tumor incidences in the 20% Miso and 4.4% NaCl groups, however, were decreased as compared with those for the groups receiving lower concentrations of Miso or NaCl. The data are in line with called 'biological sterilization' (11), a phenomenon common in radiation biology (12-17). Thus, increase in the dose of chemical carcinogenesis was associated with decrease in the incidence of tumor as found for radiation. This possibility may demand consideration in chemical carcinogenesis studies, and further studies of the mechanisms underlying such biological sterilization are clearly required.

In this study, gastric tumor incidences in the 10% and 5% Miso groups were decreased markedly as compared to those in the equivalent 2.2% or 1.1% NaCl alone groups. Cancer of the stomach has been the main subject of several epidemiological studies, in which dietary factors and particularly high consumption of NaCl or salted foods were suggested to play important roles (18-20). Experimentally, NaCl treatment was found to greatly increase the induction of glandular stomach tumors in rats (21,22). Tumors in the glandular stomach were generally increased by NaCl, but Miso at the same salt concentration caused significant decrease. Thus Miso must contain substances which inhibit induction of adenocarcinomas of the glandular stomach. We have previously reported that the soy product reduces spontaneous or fission neutron- or DEN and neutron-induced liver tumors in mice (3,4), azoxymethane-induced colon aberrant crypt foci in rats and MNU-induced mammary tumors in rats (6). Soy foods contain significant amounts of the isoflavone, genistein, which has various biological activities and antitumorigenic effects (8), as well as antiestrogenic activity (7,23). Male rats have a higher sensitivity regarding gastric tumorigenesis than females (24). We earlier found that gastric tumorigenesis was mildly inhibited by experimental treatments in initiation and promotion phases, but prevention by Miso in the promotion phase was lacking (5). Asahara *et al* (25) reported that the induction of mutation by 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) was reduced by Miso when an Ames assay was performed. It is considered that Miso's bacteria and fungi may detoxify chemical carcinogens. Koratkar and Rao (26) suggested that soy bean saponins could play an important role in inhibiting the development of ACF in the mouse colon. Saponins have also been shown to act as free radical scavengers and effectively inhibit the growth of mouse skin papillomas. Funk-Archuleta *et al* (27) reported that a soy-derived antiapoptotic facto may be beneficial as an inhibitor of chemotherapy-induced cell death in the gastrointestinal tract. Nishida and co-workers showed soy bean saponins to have an inhibitory effect on radical-initiated lipid peroxdation in mouse liver microsomes (28).

The available results thus clearly indicate that administration of Miso in the diet can inhibit the neoplastic process in several organs of experimental animals as compared with the equivalent NaCl concentration alone. Further studies are needed to elucidate the mechanism of tumor inhibition by a Miso-supplemented or -reduced NaCl diet.

Acknowledgements

This work was supported in part by a Grant from the Miso Central Institute. We would like to thank Dr M.A. Moore for critical reading of the manuscript, Ms. H. Hamada for her technical assistance and Ms. Y. Matsui for her secretarial expertise.

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