Effects of Miso in Reducing Risk of Liver and Gastric Tumors in Experimental Animals

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Summary. When intact male C3H or ^{252}Cf neutron irradiated B6C3F1 mice were fed on a diet containing 10% miso for 13 months, the frequency and multiplicity of liver tumors were significantly reduced in both cases. In irradiated females only a tendency for a similar reduction was observed. B6C3F1 male mice i.p. injected once with diethylnitrosamine (DEN) at 15 days of age and exposed to neutrons at four weeks of age also demonstrated a significantly decreased multiplicity of liver tumors when given a diet supplemented with either 10% miso or 20 ppm biochanin A (5,7-dihydroxy-4-methoxyisoflavone). In Sprague-Dawley (CD) rats treated with 100 ppm *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) for 16 weeks in their drinking water, the net incidence of gastric tumors per mg MNNG intake was reduced with a miso-supplemented diet. In addition, when six-week-old rats were given s.c. injections of azoxymethane (AOM, 15 mg/kg body weight) once a week for three weeks, and fed on diets containing 5%, 10%, or 20% miso and 10 ppm biochanin A from five weeks of age, the numbers of aberrant crypt foci four weeks after the first administration of AOM were decreased with increasing miso in a dose-dependent manner. The present results indicate that administration of a miso-supplemented diet may inhibit the development of liver and gastric tumors, as well as aberrant crypt foci, in experimental animals.

Key Words: Animals—Miso—Liver tumors—Gastric tumors—Aberrant crypt foci

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. Miso 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. In our studies, miso has been shown to facilitate the discharge of isotopes such as ^{134}Cs if given to mice prior to the administration of isotope [1]. It is also quite effective in aiding the recovery of stem cells in the small intestinal crypts after irradiation damage [2]. However, there has been almost no scientific evaluation of the biological effects of miso on human health until now. The present studies were therefore designed to examine whether the occurrence of spontaneous, radiation-induced or chemically induced liver tumors in mice, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced gastric tumors in rats, and azoxymethane (AOM)-induced aberrant crypt foci might be influenced by administration of miso in the daily diet.

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Effects of Soy Products in Reducing Risk of Spontaneous and Neutron-induced Liver Tumors in Mice

Male C3H/HeNCrj mice at six weeks of age were divided into three groups. Group 1 received the control diet (MF, Oriental Yeast, Tokyo, Japan); group 2 received a miso diet which was made into biscuits by combining 10% dry red miso provided by Miso Chuo Kenkyusho (Tokyo Japan) with 90% regular MF diet; and group 3 received a soy sauce diet which was similarly prepared by combining 10% dry soy sauce (Hiroshima Soy Sauce, Hiroshima, Japan) and 90% regular MF diet. Constituents of miso are shown in Table 1. The individual groups were composed of about 30 mice each and the observation period was fixed at 13 months after starting the experiment. The animals remained healthy throughout the experimental period and more than 90% of the mice were included in the effective numbers for the study. In the soy sauce groups, body weights (35.0 ± 5.58 g) and liver weights (1.7 ± 0.35 g) were significantly decreased as compared to those (39.3 ± 5.20 g, 2.5 ± 1.28 g, respectively) of the MF group. Miso did not influence those values, but significantly decreased the incidence (32%) and multiplicity (1.06 tumors/animal) of liver tumors as compared to those (89%, 2.86, respectively) of the control diet group (Table 2). The multiplicity (0,63) of tumors was significantly decreased in the soy sauce group (Table 2) [3].

Both sexes of B6C3F1 mice, about 30 mice in each group, were divided into eight experimental groups. Groups 1 to 4

Table 1. Composition of miso (from Miso Chuo Kenkyusho)

Composition	Dry red miso
Water	1.8%
Protein	21.0%
Fat	12.1%
Carbohydrate	38.6%
Fiber	2.3%
Ash	24.1%
Salt	21.9%
Calorie content	357 kcal/100 g

were males and groups 5 to 8 were females. Groups 1 and 5 received the MF diet for 13 months as controls, groups 2 and 6 were given 2 Gy of ^{252}Cf irradiation and the MF diet, groups 3 and 7, the 10% miso diet, and groups 4 and 8 received the ^{252}Cf neutron irradiation and the miso diet. With the neutron irradiation, average body weights were significantly decreased in males (group 3, 39 ± 4 g; group 4, 35 ± 4.4 g; compared with control group 1, 43 ± 4.8 g) but not in females. Liver weights did not show any significant differences among experimental groups, in either sex. The miso diet alone did not influence the body and liver weights in either sex. The incidence and multiplicity of liver tumors were significantly increased in neutron-irradiated males (62%, 1.25 tumors/animal, respectively) as compared to the control values (4%, 0.04, respectively), and this increase due to irradiation was significantly blocked by miso feeding (13%, 0.16). In females, while no significant differences were noted among the individual groups, a similar tendency for protection by miso was observed (Table 3) [3].

Male C3H/HeNCrj and female C57BL/6Ncrj mice were mated in our laboratory to obtain both sexes of B6C3F1 mice. At 15 days of age, experimental males were given a single i.p. injection of diethylnitrosamine (DEN) at a dose of 5 μg/g body weight. These mice were separated from their mothers at 25 days of age. When mice reached four weeks of ages they were exposed to ^{252}Cf fission neutron irradiation at a total dose of 2 Gy. They were sacrificed at 40 weeks of age. Group 1 was fed on regular MF diet throughout the experimental period. Beginning from weeks 21–28 and 32–36, mice in groups 2 and 3 were started on diet supplemented with 10% miso (group 2) and 10 ppm (group 3) or 20 ppm (group 4) biochanin A and tap water *ad libitum*. In the control and miso groups, body weights (32.8 ± 2.3 g, 32.9 ± 2.5 g, respectively) and liver weights (4.2 ± 1.3 g, 3.8 ± 1.0 g, respectively) were the same, but they were increased by 20 ppm biochanin A (35.4 ± 3.6 g, 5.1 ± 1.1 g, respectively). The incidence of liver tumors in all groups was 100%, but the biochanin A (32.9 ± 14.2 tumor number per mouse) and miso (32.5 ± 12.8) groups demonstrated decreased multiplicities of liver tumors as compared to the control values (46.0 ± 10.9, $P < 0.05$, Table 4) [4].

Table 2. Body weight and occurrence of liver tumors in male C3H mice (from [3], with permission)

Treatment	Effective number of mice	Weight (g)		Liver tumors	
		Body	Liver	Incidence (%)	Multiplicity (Number of tumors per animal)
Control	28	39.3 ± 5.2	2.5 ± 1.3	25 (80)	2.86
Miso	30	40.2 ± 3.3	2.1 ± 0.8	10 (32)**	1.06**
Soy sauce	30	35.0 ± 5.6**	1.7 ± 0.4**	12 (38)**	0.63**

** Significantly different from the control value ($P < 0.01$).

Table 3. Effects of a miso diet on the occurrence of neutron-induced liver tumors in both sexes of B6C3F1 mice (from [3], with permission)

Sex	Treatment	Effective number of mice	Weight (g)		Liver tumor	
			Body	Liver	Tumor (%)	Multiplicity (Number of tumors per animal)
Male	Control	22	43 ± 5	1.9 ± 0.3	1 (4)	0.04
	Miso	34	44 ± 6	2.2 ± 0.4	1 (3)	0.03
	Cf	29	39 ± 4[a]	1.9 ± 0.5	18 (62)[a]	1.25[d]
	Cf+Miso	30	35 ± 4[b]	1.8 ± 0.4	4 (13)[c]	0.16[c]
Female	Control	28	39 ± 6	1.4 ± 0.2	1 (6)	0.06
	Miso	31	36 ± 9	1.5 ± 0.2	0	0
	Cf	28	38 ± 9	1.7 ± 0.5	8 (29)	0.32
	Cf+Miso	24	37 ± 9	1.7 ± 0.5	3 (13)	0.13

Cf, ^{252}Cf neutrons.

[a] Significantly different from respective control values at $P < 0.05$, and

[b] also from Cf at $P < 0.01$, and

[c] significantly different from the Cf alone value at $P < 0.05$.

[d] vs [a] Significantly different from the control value ($P < 0.01$).

Table 4. Effects of biochanin A and a miso diet on the occurrence of (DEN+Cf)-induced liver tumors in male B6C3F1 mice (modified from [4], with permission)

Treatment	Effective number of mice	Weight (g)		Liver tumors	
		Body	Liver	Incidence (%)	Multiplicity (Number of tumors per animal)
Control	24	32.8 ± 2.3	4.2 ± 1.3	100	46.0 ± 10.9
10 ppm biochanin A	23	34.1 ± 2.3	4.8 ± 1.2	100	40.1 ± 10.2
20 ppm biochanin A	23	35.4 ± 3.6[a]	5.1 ± 1.1[b]	100	32.9 ± 14.2[a,c]
Miso	24	32.9 ± 2.5	3.8 ± 1.0	100	32.5 ± 12.2[a]

DEN, diethylnitrosamine.
[a] Significantly different from the control value ($P < 0.01$).
[b] Significantly different from the control value ($P < 0.05$).
[c] Significantly different from the 10 ppm biochanin A value ($P < 0.01$).

Table 5. Influence of a miso diet on the incidence of gastric tumors induced by MNNG in CD male rats (modified from [5], with permission)

Group	Effective no of animal	Total no of tumor	Gastric tumor			Small intestinal tumor	Other
			ATP	Ad-Ca	Total		
MNNG + 10% Miso	20	12 (60)	3 (15)	4 (20)	7 (35) *1.48*	6 (30)	0
MNNG + 2% NaCl	20	14 (70)	5 (25)	5 (25)	10 (50) *2.27*	7 (35)	1 (5)
MNNG + MF	20	7 (35)*	3 (15)	2 (10)	5 (25) *1.32*	3 (15)	0

MNNG, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; ATP, atypical hyperplasia; Ad-Ca, adenocarcinoma; *Italics*, net incidence of gastric tumor/mg MNNG intake.
* Significantly different from MNNG + 2% NaCl ($P < 0.05$).

Effects of Miso Diet or Sodium Chloride Diet on Gastric Tumorigenesis in Rats [5]

Five-week-old male CD(SD):Crj rats were treated with 100 ppm MNNG for 16 weeks in their drinking water. They were maintained on diet supplemented with 10% dry miso or 2.2% sodium chloride, or the MF control diet, from five weeks of age to the autopsy time point at 52 weeks. The intake of MNNG in the miso group (23.6 ± 2.0 ml/animal per day) was significantly elevated as compared to the NaCl group (22.0 ± 2.6 ml, $P < 0.05$) and MF group (18.9 ± 2.1 ml, $P < 0.05$), while the intake of diet in the miso group (18.4 ± 1.4 g/animal per day) was significantly more than in the NaCl group (17.8 ± 1.8 g, $P < 0.01$). Body weights did not significantly differ among groups. Liver weights in the NaCl group were significantly lower than those in the Miso and MF groups. Gastric tumors developed in 50% of the NaCl group, 35% of the miso group, and 25% of the MF group. There were no significant differences in gastric tumor incidences among the groups but taking into account the increased intake of MNNG in the miso group, the net incidence of gastric tumors per mg MNNG intake was decreased in the miso group as compared with the NaCl group (Table 5).

Table 6. Inhibition of aberrant crypt foci induced by AOM by a miso diet in F344 rats

Group	Number of animals	Number of ACF	Mean number of crypts/focus	Total crypts/colon
Control	3	0	0	0
AOM	7	155 ± 53	1.66 ± 0.77	253 ± 74
AOM + 5% Miso	10	134 ± 52	1.83 ± 0.88	240 ± 93
AOM + 10% Miso	10	86 ± 70*	1.94 ± 0.96	163 ± 131
AOM + 20% Miso	10	66 ± 41**	1.88 ± 0.91	124 ± 78**
AOM + biochanin A	7	132 ± 63	1.81 ± 0.83	227 ± 90

Y. Masaoka and H. Watanabe, unpublished data.
AOM, azoxymethane; ACF, aberrant crypt foci.
* Significantly different from the AOM alone value ($P < 0.05$); ** Significantly different from the AOM alone value ($P < 0.01$).

Inhibition by a Miso Diet of AOM Induction of Aberrant Crypt Foci

The modifying effect of a miso diet on the induction of AOM-induced colonic aberrant crypt foci (ACF) [6] was investigated in male F344 rats. Animals were given weekly s.c. injections of AOM (15 mg/kg body weight) for three weeks to induce ACF. These rats were fed diets containing 5%, 10%, or 20% miso or 10 ppm biochanin A for five weeks, starting one week before the first AOM dosing. All rats were killed two weeks after the last AOM injection, to assess the numbers of ACF in the large intestine. In rats given AOM, the number of ACF/large intestine was decreased in a dose-dependent manner ($y = 150 - 45.9x$, $r = -0.95$) by miso but not biochanin A. There were no significant differences in numbers of ACF/focus or total number of crypts/large intestine among groups (Table 6). However, when dimethylhydrazine (15 mg/kg body weight 10 times over a 10-week period) was given to CD-1 male mice receiving an MF or miso diet, and animals were killed 10 weeks after the last treatment, there were no significant differences in colon tumor incidence, number of tumors per animal, or tumor size [7].

Mechanisms of Tumor Inhibition by Miso

In the presently described studies, soy bean products reduced the risk of liver tumors occurring naturally, induced by neutron irradiation or by a combination of DEN and neutron irradiation. There have been numerous reports of factors promoting liver tumors in B6C3F1 mice, including radiation and medicines [8,9]. On the other hand, there is evidence that restricted diet prevents or inhibits liver tumor development in mice [10]. Even intermittent administration of miso or biochanin A reduced liver tumor multiplicity, indicating that continuous administration of these compounds may not be a necessity for tumor inhibition. After two weeks of exposure, biochanin A, which is a component of miso, was able to suppress DNA synthesis in adult mice [4]. This is in line with work by Yanagihara et al. [11], who reported that the growth of human gastric cell lines cultured in vitro is greatly inhibited by addition of genistein or biochanin A, and suggested that the mechanism of action was apoptosis. Isoflavone in soy bean has also been reported to have an antioncogenic effect [12] and genistein, a member of the isoflavone family, has been found to inhibit topoisomerase activity and growth of NIH 3T3 cells [13]. Moreover, Asahara et al. [14] reported that the induction of mutations by Trp-2 was blocked by miso when an Ames assay was performed. It has also been reported that soy bean administration inhibited the occurrence of breast carcinomas induced in rat by methylnitrosourea (MNU) [15]. Gotoh et al. (unpublished data) found that miso and/or Tamoxifen decreased the incidence and multiplicity of mammary tumors in MNU-treated SD rats. The available results thus clearly indicate that administration of miso in the diet can inhibit the neoplastic process in several organs of experimental animals. Further study is needed to elucidate the mechanism of tumor inhibition by a miso-supplemented diet.

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