Miso and its biological effects
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**Introduction**

A traditional ingredient of the Japanese diet, miso (fermented soy bean paste), is fermented from soybeans, rice, wheat, or oats. It contains vitamins, microorganisms, salts, minerals, plant proteins, carbohydrates, and fat. Miso also contains saponin inhibiting lipids peroxide, trypsin inhibitor, isoflavon, lecithin, colin, prostaglandin E and others[1]. It is used on a daily basis as a flavor in soup and solid food in Japan and other parts of Asia and remains an essential ingredient for Japanese-style cooking. Even though miso has no equal in the West, Western cooks familiar with miso prize it for its almost unlimited versatility. It can be used like bouillon,as a rich meat stock in soups and stews[2]. It is considered as a food with health-promoting benefits, such as effectiveness in relieving fatigue, regulation of the intestinal function, digestive supplement, protection against gastric ulcer, decrease of cholesterol, decrease of blood pressure, whitening ability, prevention of diseases associated with adult lifestyle habits, apoplexia cerebri, accumulation of brain metabolism, protection of aging, healing radiation damage and prevention of cancers for biological effects.

In this review, the effects of miso for radiation prevention, cancer prevention and prevention of hypertension were described with a focus on epidemiological and experimental .tests.. .

**1. Radiation protection by miso**

In August 9th, 1945, the 2nd atomic bomb was dropped on Nagasaki. At the time, physician Tatuichirou Akizuki, worked with 20 employees, caring for 70 tuberculoses patients in" Uragami Daiichi Hospital" (St Francis Hospital) located about 1.4km away from the hypocenter. However, these people, including Dr. Akizuki, escaped from death caused by acute radiation damage. Dr. Akizuki conjectured that the reason there was no nuclear bomb disease was that these people had consumed cups of wakame miso soup (miso soup with garnish of wakame seaweed)[3] everyday. Later, his assumption was published in the English language for the information of the Western population. On April 26, 1986,after the accident at Chernobyl, Russia, many Europeans consumed miso soup to prevent radiation diseases. In effect, Dr. Akizuki was the first person in Japan to promote the discovery of the preventive characteristics of miso against radiation damage and also its contribution to a healthier life among the modern generation.

Based on this case of Dr. Akizuki, our experimental studies were started to research the protective effect of miso against radiation damage.[5-7]

A week before irradiation, five-week-old male B6C3F1 mice were fed a commercial diet of MF (common rodent chew, Oriental Yeast, Tokyo) and MF with 10 % dried red rice miso obtained from Miso Central Institute, Tokyo. The mice for 6 weeks were irradiated with 7 – 12Gy (dose rate 4 Gy/min) for small intestinal crypt survival and were not anesthesized during the irradiation. An histological autopsy was performed on the animals for observation of crypt survival 3.5 days after irradiation.

No radiation protection was shown on 2% of NaCl or MF diet (common rodent diet) groups but the numbers of surviving crypts were significantly increased on miso group. No protective effects were evident when miso was given immediately, 1 or 2 days after irradiation. We concluded that fermented substances might protect not only against gastrointestinal damage but also bone marrow death by radiation. Therefore, it was shown that the protection effectiveness required minimum concentration of the preventive substance(s) at certain level in blood by long term intake of miso[4].

An investigation was then conducted to prove whether the protection effect was caused by soybean alone or fermentation periods of miso. One week before irradiation, the mice were given a diet supplemented with 10% of miso at different stages of fermentation such as 3 to 4 days, 120 days and 180 days fermented. After 7 Gy, the irradiation number of surviving crypt was significantly greater for the 120 days fermented miso and 3 to 4 days fermented miso as compared to the MF group. With 8 Gy, surviving crypts in the 3 to 4 days and 180 days fermented miso groups were also significantly increased as compared with the MF values. Crypt survival was significantly different in the 180 days fermented miso group ($p<0.01$) as compared with the 120 days fermented miso and MF groups (Table 1). The lengths of surviving crypts with 7 and 8 Gy irradiation were increased as compared with those at 0 Gy. Crypt survival was significantly different in the 180 days fermented miso as compared with 3-4 days fermented, 120 days fermented miso and MF groups[6-7].

By using miso produced by Miso Central Institute (Tokyo) or Hiroshima Prefectual Food Technology Research Center, crypt survivals were studied at the

dose of 12Gy X-irradiation. There were no significantly different numbers of surviving crypts on both 180 days fermented miso groups.   In different types of miso provided from different areas of each institutes, longer fermented miso increased the number of survival crypts and the period of 180 days fermentation is considered more significant to radiation protection.   The mechanism of radioprotective effect of miso is considered to be closely related to the substance produced during fermentation stages[8].   Thus, the radiation protection effects of miso might be attributed to the fermentation process.

The, survival rate of the animals at the dose rate of 4 Gy/min did show that there was no difference in groups of mice irradiated at the dose rate of 2 Gy/min. With 15Gy, animals started to die on the 5th day. On the 7th day, all animals were dead and s no significant difference was observed.   But with 8 Gy at 10th day, MF group stared to die on the 5th day.. At 11th day, The 120 days group started to die on the 5th day and the 180 days group on the 13th day.   Using the Cox model, results proved that the 3-4 days ($p<0.048$), 120 days ($p<0.026$), and 180 days ($p<0.011$) groups that were fed the fermented miso had a significantly longer survival period than the MF group. Therefore, at high dose irradiation, the death of the animals was   inevitable; but at low dose, after 180 days, the fermented miso has an effect of radiation protection[6].   We can conclude that the fermented substances protected not only against gastrointestinal but also bone marrow death by radiation.   Houchen et al[9] reported that expression of FGF-2 is induced with radiation injury and recombinant human FGF-2 markedly enhanced crypt survival. Takahama et al[10] also reported that a replication-deficient adenovirus containing the HST-1 gene acts as a potent protector against lethal irradiation associated with injury to the intestinal tract as well as myelosuppression in the bone marrow and spleen.   Ferrel et al[11] have reported that recombinant human keratinocyte growth factors can protect mice from chemotherapy- and radiation-induced gastrointestinal injury and death but not against whole-body radiation, at least in terms of death from intestinal and contaminated marrow.   We also found VEGF to have protective Katoh and Watanabe,( unpublished data).   Cytokine-like substances in miso may thus play an important role in the prevention and/or the recovery and repopulation of critical tissue elements when given prior to and during radiation exposure. However, to our knowledge there are no reports regarding extraction of cytokines from miso[9-11].

Furthermore, we are conducting a search for effective materials.   Water soluble

and ether soluble fraction as well as the residue from 10 days and 260 days fermented miso were investigated.   Only water soluble fraction of 10 days miso has no effect on crypt survival but the rest of the fractions of 10 days and 260 days did have an effect on crypt survival.   As for mortality, only water soluble faction of 260 days miso increased survival rate but the rest of each fraction of 10 days and 260 days had no difference compared with control (MF).   This suggests that the effective substances in this fraction might be synthesized or the preventative   substance in 10 days miso was produced during 260 days fermentation time[8].

On the other hands, aglycon type isoflavon and melanoidin were produced during fermentation of miso, so radiation protection of those substances was studied.   Aglycon type isoflavon did not have any radiation protective effect. (Watanabe et al. unpublished data) but chemically synthesized melanoidine and the melanoidine-like substance extracted from 7-month fermented miso did affect radiation protection.   Therefore, melanoidine like substances in 180 days fermented miso might be considered to have radioprotective effect[12].   Further study is needed to discover the substances responsible for increased crypt survival, crypt lengths and prolongation of average time to death with miso, their mechanisms and any associated changes in bacterial flora in the gastrointestinal tract.


**2. Prevention of gastric tumor**
**2.1 Effects of salt in miso**
Hirayama reported that consuming miso soup everyday reduced the occurrence of stomach cancer[13].   On the other hand, as miso contains 10- 12 % salt content, it has been considered that high consumption of NaCl greatly increased the occurrence of stomach cancer[14].   This chapter discusses the difference beween salt in miso and salt itself.

Male CD:Crj rats were fed $N$-methyl-$N'$-nitro-$N$-nitrosoguanidine (MNNG) in their drinking water for four months.   The rats were simultaneously maintained on a diet supplemented with 10 % dry red miso, 5 % red dry miso, 2.2% NaCl since 10% dry miso contains the equivalent amounts of 2.2% NaCl or 1.1% NaCl and 5 % miso contains 1.1% NaCl or control MF feed.   As control, MF in stead of MNNG, tap water is given during diet[15,16].   Generally, the incidence of gastric tumors of NaCl groups was higher than miso groups. especially, the

incidence of gastric tumors of the MNNG+2.2% NaCl. The size of gastric tumors in the MNNG+2.2% NaCl and MNNG+1.1% NaCl groups and adenocarcinomas in MNNG+1.1% NaCl groups were significantly increased as compared with MNNG+MF. (Table 2)

Furthermore, the same experiment was conducted on 50% salt-reduced miso but the incidence of gastric tumor in the   reduced salt content miso (1% of NaCl content) was the same as the regular miso containing 2% NaCl[17].   This study indicated an increased preventative effect on gastric tumor by miso than by salt diet.   Asahara et al.[19] and Rajendran et al.[20] reported that by Umu test to indicate mutagenicity, the strains of yeast, lactic acid bacteria and other molds in miso can remove or detoxicate carcinogens as trp-p-2 from burnings of foods. Yanagihara et al [21] reported that by using digestive tract cells, a group of isoflavone-like substance in miso such as biochanin A and genistaine prevented the growth of gastric tumor cells and caused   the destruction of the cells by apotosis.

These reports suggested that substances contained in miso desroyed or degenerated carcinogens to decrease the carcinogenicity of MNNG.

## 2.2 Effects on different fermentation stages of miso
To determined whether soybean themselves or the fermentation process produced the preventive agents,, a study was conducted to examine the effects of miso at various fermentation-stages on the initiation phase of MNNG-induction of rat glandular stomach. tumors.

Male CD:Crj(SD) rats were maintained on MF diet supplemented with 10 % of dried miso prepared from three different fermentation stages such as 3 to 4 days, 120 days and 180 days with.1% NaCl (Wako, special grade) feed as control. During administration of carcinogen, the animals were fed with the above diets. [22].   (Table 3)   There was no significant difference among groups in total number of tumors, number of tumors per rat, incidence of gastric tumors, duodenum tumors and other tumors.   For gastric tumors,the size of the tumors in 180 days miso group decreased significantly compared with control (P<0.01). Size of tumors in 3-4 days fermentation miso group were significantly increased as compared with 120 days fermented miso (P<0.05) and 180 days fermented miso (P<0.01).   There is a high possibility that during fermentation period of

miso, the substances to inhibit growth of tumors might be produced.

During fermentation of miso, two reactions occur: enzymatic reaction by koji (rice starter with *Aspergillus oryzae* ) and fermentation reaction by microorganisms as yeast, lactic acid bacteria.   By enzymatic reaction, soybean and rice were decomposed and from hydrolysis of protein, saccharification of starch and decomposition of oil, low molecular peptide, amino acids, reduced sugars, fatty acids and glycerol were produced.   A part of these nutrients were consumed by salt-tolerance microorganism, as a by-product of metabolism of fermentation. Alcohol, organic acids and esters, flavor components of miso, were synthesized. In addition, maillard material was produced by non-enzymatic reaction and the material is a factor of color in miso[2].   Fujinami et al. reported that content of glutamine and formol nitrogen were increased during miso fermentation[22] and Higashi et al. reported the production of amylase and acid protease during the fermentation period[23].   These compounds might be produced from fermentation as well as the soy bean..   However, analysis of these compounds is for future investigation.


## 3. Prevention of aberrant crypt foci and of large intestinal cancer

The recent change of dietary habits from traditional Japanese to Western style diets has been paralleled by an increase in colon and beast cancer in Japan. The change is more responsible for the increase in cancer than genetics.[24]    It was reported that an epidemiologic study showed cancer of the large intestine was suppressed by soy bean[25].   Many articles had been published concerning physiological property of soy bean inhibiting large intestinal cancer or aberrant crypt foci (ACF) in the aspects of vegetable fiber, [26] isoflavon, [27-29] saponin[30] and high molecular weight fraction of water soluble material[31,32] of soybean. There also are articles to describe the non-effective aspect of soy bean on cancers[28,29].


### 3-1. Effect of miso, salt, kojic acid and biochain A on ACF

The study was designed to investigate the effects of fermented miso, pure NaCl, crude salts, koji acid and biochanin A in the diet on the induction of aberrant crypt foci (ACF) by azoxymethane (AOM), a carcinogen for large intestinal cancer[33-35].   (Table 3)

Six-week old male F344/DuCrj rats were fed on each test diet.. After one week, AOM (Sigma) was injected by 15 mg/ kg/body once per week for 3 weeks and the rats continued to be fed different diets for 2 weeks.   All rats were fed a commercial diet MF alone or with 5%, 10%, 20% of dried red miso, 2.2 % and 4.4% of NaCl (Wako Pure Chemical) which are equivalent NaCl content of 10 % and 20% miso respectively, 4.4% of sea salt (Ryoen, Tokyo), coarse salt (Diasalt, Tokyo), rock salt (Tomen, Tokyo), table salt (JT, Tokyo) and 1.5 % or 3.0% of kojic acid (Nagase Seikagaku), 5 ppm of biochanin A (Sigma).   After the animals were euthanized, the colon was removed and fixed in 10% buffered formalin and stained with 0.5% methylene blue. By viewing the stained colons with a light microscope at magnification of 20-30 times, the numbers of ACF, total number of aberrant crypt (AC) per colon and of AC per focus were counted.

All rats treated with AOM showed 100% incidence of ACF. The number of ACF per animal was significantly decreased except in the groups of 4.4 % pure NaCl, 2.2 % pure NaCl group, 5 % miso and biochanine A.   The number of total AC significantly decreased except in the group of 2.2% of pure NaCl, 3% kojic acid and biochanine A. AC per focus increased significantly in the 10%, 20% miso group.

ACF decreased as concentration of miso increased but not by the pure NaCl. ACF decreased in the group of crude salts and 3.0% of kojic acid but not in the group of 1.5 % kojic acid and biochanine A.   Kojic acid exists in miso during fermentation period but does not in the final product. (Mohri & Fujinami, personal communication). Therefore, the suppressing effect on ACF of miso did not appear to be kojic acid nor similar substance.

This study showed pure NaCl did not have a suppressing effect on ACF but crude salt did.   Some of the groups were measured by BrdU labeling index or germinal regions but there was no correlation between ACF and AC induction. Impurities of purified salt used in this study were less than 0.01% but crude salt contained calcium and minerals and these substances were reported to suppress cancer in the large intestine and ACF induction [36-41] while also preventing DNA synthesis [42,43].   It is also reported that seren [46], copper and iron [47] had the same effect of suppression.   Therefore, suppression effect of ACF induced by AOM was considered to be caused by the minerals in miso such as calcium and

magnesium. This fact suggests that large intake of minerals in sea salt used in Japanese regular food, is a factor in lowering the occurrence of large intestinal cancer among Japanese people.

### 3-2 Effects of long term fermented miso on ACF

The study was designed to investigate the effect of different fermentation times of miso on the ACF induced by AOM[46].

All rats treated with AOM developed ACF. The number of ACF per animals were, 87.8±28.9 in group of MF, 84.9±52.1 in 2.2% NaCl, 85.5±19.3 in short-term (3-4 days) fermented miso, 83.6±20.8 in middle-term (120 days) fermented miso, 65.1±18.4 long-term (180 days) fermented miso. The number of ACF in long-term miso decreased significantly compared with MF and short term, and middle term groups. Total AC number in middle-term miso was largest and increased significantly compared with 180 days miso. In this study, long term fermented miso was the most effective suppresser of ACF induced by AOM. The effect was considered to be caused by components of soy bean, minerals, and other substances produced during fermentation of miso as previously described.

### 3-3. Effect on large intestinal adenocarcinomas

Five-week-old male F344/DuCrj rats were maintained on MF diet supplemented with 10 % of each dried miso prepared from two different fermentation stages such as short term (3 to 4 days) and long term (180 days). Twenty-four weeks after administration of carcinogen AOM, the animals were euthanized and the colons were investigated. There was no statistical difference among the groups in averaged number of ACF per colon, total number of AC and number of AC per ACF[47-48]. (Table 4)

Each animal that died on 103rd, 149th and 163rd day after the first AOM injection in miso groups had signet cell carcinoma in the duodenum. By pathological observation, signet ring cell carcinomas in the large intestine were not observed in the group of 180 days fermented miso. The size of well-differentiated types of adenocarcinomas and total adenocarcinomas (well-differentiated type and signet ring cell) in the 180 days and 3-4 days fermented miso groups was significantly smaller in MF+AOM group. The

number of crypts cell, PCNA-positive cells, the highest position and width of the germinal region of positive index, decreased significantly in 180 days miso compared with MF+AOM values.   In the present study, dietary administration of 180 days fermented miso inhibits the development of AOM-induced ACF in the rat colon. But 10 % miso did not have the suppressive effect on ACF induced by DMH[50].

## 3-4. Effects of soybean products on colon carcinogenesis

Epidemiological study showed that intake of miso soup decreased large intestinal cancer[50],[51]   Kono et al reported that taking two cups of miso soup reduced the   amount of S type colon cancer.[52].   By taking tofu and soybean, Watanabe et al reported a decrease in rectal tumors[53] and Poole et al reported a decrease in large intestinal tumors[54]
caused by soybean products and miso. Hu et al reported a decrease in colon tumors of men in China[55].   LeMarchard et al reported, intake of soybean or soybean products in Hawaii reduced occurrence of large intestinal cancer in women but not in men[56].   Tuyns et al reported soybean to be clearly a preventive agent against colon rectal cancers on the basis of a case control study in Belgium[57].

In the study by Davies, F344 rats were fed on soybean with high content isoflavon and on a diet supplemented with high fat and with less calcium one week before and 31 weeks after AOM administration.   The soybean group showed no decrease in the occurrence of large intestinal tumors[58].   Min mice were fed a Western type of diet (high fat and less vegetable fibers and calcium) with higher amount of isoflavon added, but the study did not show any preventive effect on large intestinal cancer[59].   Rao[60] reported the increase in non-invasive tumor numbers and adenocarcinomas based on the study of AOM-treated rats fed by casein-based feed with genistaine.   McIntosh[61] reported a greater increase in the number of tumors with soy protein than casein. Gee [62], using a DMH model, reported no suppression of ACF in large intestinal tumors by genistein. l.   Hahhak et al[63] and Thiagarajan[64] observed a suppressive effect of soy or soy products against ACF induction.   Pereira et al[65] also found purified genistein to inhibit induction of ACF.   Since isoflavon has anti-estrogen activity, it is considered to be a most effective substance against breast cancer[66-68].   Gotoh et al demonstrated that administration of

biohcanin A, a genistein precursor, inhibited the development of mammary tumors but we reported biochanin A did not reduce colonic ACF development in rats treated with AOM[69].    It is considered that isoflavon has a preventive effect on cancer of breasts and internal organs related to hormone. There is no clarification of the epidemiological relationship between large intestinal cancer and isoflavon.

In the present study, long-term as 180 days fermentation miso significantly decreased in the PCNA positive index.   It is hypothesized that calcium is a regulator of cell proliferation in the colon. Dietary intake of calcium inhibits experimental carcinogenesis[70].   Therefore, during fermentation of miso, the substance to suppress cell proliferation is generated and it might work as a factor in preventing colonic cancer

## 4. Prevention of lung cancer

In Japanese, lung cancer has exceeded stomach cancer and become the highest incidence of cancer.    Since it became a serious problem, it is an urgent matter to develop a preventive method of lung cancer.   Epidemiological studies in Okinawa[71] and Singapore[72] have indicated that fermented soy-bean products might have inhibitory effects on lung cancer. Shiraki[73,74] investigated such effects of fermented miso by using short-term fermented as 3 -4 days, and long-term as 180 days miso.

Six-week old male Slc:Wister rats were treated di-isopropanol-nitrosomine (BHP) in drinking water at concentration of 200 mg/liter for 10 weeks.   All rats were fed by commercial diet MF (Oriental Yeast Co., Ltd. Tokyo) alone or with added miso.   Short-term dry red miso, 3-4 days, and long-term, 80 days, fermentation (Miso Central Institute, Tokyo) were   supplemented into MF at 10%.   The diets were supplied with normal tap water *ad libitum* for 12 weeks after treatment of BHP and the rats were euthanized 22 weeks after the treatment. Macroscopically, all animals had many nodules in the lungs.   The number of nodules in the 180 days fermented miso group was significantly lower as compared to other groups but the size of tumors did not show significant difference among groups.   All animals had hyperplasia, adenomas and adenocacinomas. Papillomas or squamous cell carcinomas were also evident in

13%, 27% and 29% of the animals in the BHP, BHP+short, BHP+long but rate of incidence had no significant different. numbers of adenocarcinoma in the BHP+long and PCNA. Strongly positive tumors in the miso group were significantly decreased as compared to the BHP alone group. Size of tumors decreased significantly in BHP+long group. It was obvious that the long-term fermented miso reduced the numbers of lung tumors. (Table 6)

Koo et al[75] reported a significant inverse association between tofu/soy intake and lung cancer in non-smokers in Hong Kong after adjustment for age, numbers of live birth and schooling. Swanson et al[76] reported a dose-dependent inverse relationship with tofu and risk of lung cancer in Yunnan Province, China. On the other hand, Ozawa et al reported that among females, a high intake of miso-soup significantly and almost dose-dependently increased the risk[77] However, consumption of miso-soup seems to be associated with Japanese-style diet and increased salt intake. But, the significance of these finding is unclear.

**5. Prevention of hepatic tumor**
When C3H male mice were bred for 1 year under normal condition, 89% of the mice had natural occurring liver cancer. When B6C3F1 mice were irradiated by $^{252}$Cf neutron, which is the same neutron of atomic bomb, 2Gy. Thirteen months later, 62% of the male mice and 29 % of female had liver cancer. However, these mice were fed on diets containing 10% miso (Miso Central Institute, Tokyo), the frequency of cancer of C3H, male B6C3F1 and female B6C3F1 was reduced to 32%, 13%, 13% respectively[78].

Ogundigie et al demonstrated the same effect of miso. Strong carcinogen for hepatocytes, diethyl-nitrosamine (DEN), was injected to 15 days-old B6C3F1 mice and at 4 weeks-old, the mice were irradiated at 2Gy of $^{252}$Cf neutron and at 40 weeks-old, the animals were euthanized for the investigation. Various kinds of preventive materials including miso (Miso Central Institute, Tokyo), were applied to find out occurrence of hepatic tumors. All animals had hepatomas and control groups without any preventive material had 46 tumors/mouse. But in case of miso, number of tumors reduced to be 32.5/mouse significantly. When 10 ppm and 20 ppm of biochanin A were applied, the numbers of 10 ppm were 40.1/ mouse but the number of 20 ppm reduced significantly to be 32.5/mouse[79].
Obviously, both results indicated that the administration of miso inhibited the development of hepatomas induced by natural or $^{252}$Cf neutron or carcinogen in

experimental animals.

Kurosawa et al[80] reported that miso-soup among women without history of liver diseases showed a significant inverse association with hepatocellular carcinoma mortality.  And also Sharp et al[81] suggested that consumption of soy foods especially miso-soup, is associated with reduced risk of hepatocellular carcinoma.

## 6. Prevention of breast cancer

Yamamoto et al[82] reported that taking a cup of miso three times a day had reduced the occurrence of breast cancer but   tofu, natto, soybean and fried bean curd did not have such effect and women in menopause had less breast cancer when a large amount of isoflavon was taken.

Baggott el al reported that when dimethylbez{a}-anthracene (DMBA) was administrated to Sprague-Dawley (SD) rats, 4.1 per rats of mammary tumors occurred but when the animals were fed on diets containing 25% miso, the numbers of tumors were reduced to be 2.95 per rat and the time until occurrence of tumor with miso became longer than with only carcinogen[83].

Gotoh et al[69,84] investigated the induction of mammary tumors by administration of soybean, miso, and biochanin A in 40mg/kg of $N$-nitoro-$N$-methylurea (MNU) treated SD rats.   The incidence time of the tumors was slower in miso and biochanin groups than MNU itself. The number of tumors was 2.2 in MNU but in miso group, it significantly reduced to be 1.2 and also  in  10 % soybean and biochanin A groups.   They also investigated the incidence of tumors by miso and tamoxifen, a medicine used for breast cancer treatment. The number of tumors per rat induced by MNU only was 4.5 but it was 2.4 in miso groups and it was 1.4 in tamoxifen groups and the number was significantly reduced to be 0.2 when miso and tamoxifen were fed together. This investigation clearly indicated that administration of miso in the diet can reduce the occurrence of mammary tumors as well as tamoxifen at the beginning of carcinogen treatment and it can also suppress the multiplicity of tumors induced by carcinogen.

## 7. Protection against hypertension.

It was often considered that salt in miso induced stomach cancer[14] as well as the

increase in blood pressure[85-91] and even excess amount of intake of miso led to lifestyle-related disease. As described before, it is unlikely salt itself or salt in miso did not cause the increase of gastric tumor.　Afterwards, the effects of salt in miso on blood pressure were studied[92].

Six-week-old salt-sensitive Dahl rats or similar SD male or female rats (original strain of salt-sensitive Dahl rat) were maintained on commercial diet MF alone or supplemented with 10 % dry red miso (180 days fermented miso, Miso Central Institute, Tokyo, Japan) or 2.3% salt because 10% dry miso containing 2.3 % NaCl or 1.9 % salt or 0.3 % salt for 12 weeks. Systolic blood pressure in the Dahl male rats on 2.3% NaCl was significantly increased for 8 and 12 weeks but not in SD rats.　However, the blood pressure on diet with miso or commercial control diet (MF) was not increased. Even though miso contains 2.3% NaCl, the blood pressure was stable as on commercial diet containing 0.3% salt.　(Table 7)

Systolic blood pressure of Dahl female rats was increased on salt diet but not increased on miso diet.　The blood pressure of female rats was not increased on 1.9% salt diet but that of male was increased. Male rats are more sensitive to salt than female rats similar to human beings. (Table 8)

As a result, blood pressure of salt-sensitive rats was not increased on miso diet that included 2.3% NaCl. This indicated that these rats are regarded as salt-sensitive as human beings. It was concluded that the miso diet did not increase blood pressure in such people.　Salt in miso worked in different manner from salt itself for gastric tumors as well as blood pressure. Considering the result of blood pressure between salt in miso and salt itself, it is better to refrain from taking a lot of salt. Rather it is recommended to take miso soup more often to preventthe occurrence of lifestyle-related disease.

　In epidemiological data, He et al demonstrated that dietary soybean protein reduced blood pressure in a randomized, double-blind, controlled trial in Chinese adults[86,87].　Several small clinical trials have reported inconsistent findings regarding the effect of soybean protein on blood pressure[91,94,95]. Washburn and colleagues[91] compared the effect of 20 g of soybean protein containing 34 mg of phytoestrogens given either in 1 dose or in 2 doses with that of 20 g of complex carbohydrates on cardiovascular disease risk factors and menopausal symptoms among 51 women in a randomized, controlled trial.

They observed a significant reduction in diastolic blood pressure in the twice-daily soybean protein diet compared with the carbohydrate control diet. Burke and colleagues[94] examined the effects of soybean protein on 24-hour ambulatory blood pressure among 41 treated hypertensive patients in a randomized, controlled trial. He also documented that blood pressure reduction associated with soybean dietary protein was greater than that of the carbohydrate control[92]. Conventionally, increased intake of complex carbohydrate has been recommended as a replacement for saturated fat intake to reduce cardiovascular risk[96]. Soy-based diet attenuated the development of hypertension both in female and male SHR rats[97]. These findings suggested that increased intake of soybean protein may play an important role in prevention and treatment of hypertension.

Nakamura et al[97] conducted a double-blind, randomized placebo-controlled study to evaluate the effects of 6-week diet containing approximately 25% to 20% low-sodium soy sauce and miso (an approximately 10% reduction of total dietary salt intake) in Japanese on blood pressure. The changes in blood pressure observed in the experiment were not significant. However, in those aged 40 years and older, a 6.4mm Hg net reduction in diastolic blood pressure with no significant change in systolic blood pressure was noted in the low-sodium intake group. Nakamura et al reported that a 6-week trial of low-sodium soy sauce and miso resulted in a reduction in urinary salt of 0.7g/day in the intervention group and the net difference of urinary salt excretion between the intervention group and the controls was 1.4g/day. They concluded that an approximately 10 % reduction of dietary salt intake affected diastolic blood pressure in middle-aged or older people. When one consumes 13g of dietary salt daily, the estimated salt intake from soy sauce will change from 3.3g to 2.4g and that from miso will change from 1.6 to 1.3 by replacing the seasoning with the low-sodium type.Thus one can achieve 1.2g reduction of daily dietary salt intake. Dietary reduction of NaCl in soy sauce rather than miso may be used to reduced diastolic blood pressure. On the other hand, Kanda et al performed a study of the association of lifestyle parameters with the future risk of hypertension in normotensive subjects. They administered a baseline questionnaire and performed a four-year follow-up of 445 normotensive Japanese from 35 to 89 years of age. In 60 to 69 year old subjects, changes of blood pressure during four years were negatively correlated with boiled rice intake in men and with Japanese tea intake in women[98]. Multiple logistic regression analysis revealed that miso-soup intake of two or more bowls per day

protected against hypertension during the follow-up (p < 0.05). These results indicate that the nature and amounts of food intake is important in the prevention of hypertension in the elderly. It also prevents increased blood pressure when compared with rats fed the equivalent NaCl concentration without miso in men and animals. For protection of hypertension, Fujita recommended[99] that taking miso soup together with protein or together with potassium rich vegetable as spinach or wakame seaweed prevented the increase in blood pressure even in salt sensitive persons.


**Conclusion;**

Highest occurrence of cancer used to be stomach cancer in Japan. However, the recent change of dietary habits from traditional Japanese to Western style increased the occurrence of cancer of large intestine, breast cancer and prostate cancer more than stomach cancer. This indicates the importance of dietary habit and traditional dietary habits as miso soup should be re-evaluated not only in Japan but in the world. Since miso has such functions as discussed in previous sections for preventive effect on maintaining your heath, further studies are needed to clarify the mechanism and effective materials produced during fermentation in miso. As a whole, miso is considered to be a good food ingredient for health.

Finally, the effective substances caused during fermentation are unclear. Kennedy[100,101] have declared that soybean-derived Bowman-Birk inhibitor suppresses carcinogenesis. This substance should be one of the candidates for analysis of biological effective compounds in miso. This is for future investigation.

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Table 1　　Number of surviving crypts after X-irradiation[6]

|  | 0 Gy | 7 Gy | 8 Gy | 10 Gy | 12 Gy |
|---|---|---|---|---|---|
| MF（Control） | 123.7±13.1 | 97.6±10.8 | 80.8±8.9 | 48.7±7.1 | 30.3±5.8 |
| 3-4 days fermented miso |  | 112.5±14.0[**] | 97.3±13.7[**] | 53.0±6.4[*,a] | 41.0±6.5[**,a] |
| 120 days fermented miso |  | 108.4±13.9[**] | 84.4±11.7 | 55.1±5.4[**,a] | 43.9±5.1[**,a] |
| 180 days fermented miso | 125.2±12.7 | 103.0±11.6 | 87.4±9.1[**] | 68.5±9.3[**] | 50.0±5.2[**] |

(Mean±SD)

[**]: Significantly different from MF value ($p < 0.01$)

[a] ：Significantly different from 180 days fermented miso value ($p < 0.01$)

Table 2　Incidence of gastric tumor by miso and NaCl by *N*-methyl-*N*'-nitro-*N*-nitorosoguanideine (MNNG) in CD(SD) rats[15]

| Group | Total tumor (Number per rat) | Glandular stomach | | | Small intestine | | | Other tumor |
|---|---|---|---|---|---|---|---|---|
| | | Incidence | Size (mm) | Number | Incidence | Size (mm) | Number | |
| MNNG+10%Miso | 17/20(85.0%)a (1.15±0.75)a | 9/20(45%) | 2.3±3.5 | 0.6±0.7 | 9/20(45%) | 8.2±13.9 | 0.6±1.0 | Sarcoma |
| MNNG+2.2% NaCl | 17/19 (89.5%)a (1.32±0.67)a | 13/19(68%)a | 4.1±5.1a | | 11/19(58%)a | 11.4±17.1 | 0.6±0.8 a | Squamous cellcarcinoma Plasmacytoma |
| MNNG+5%Miso | 10/19 (52.6%) (0.63±0.68) | 7/19(37%) | 2.0±2.9 | 0.5±0.7 | 2/19(11%)b | 5.0±17.5 | 0.2±0.5 | Lymphoma Hepatoma |
| MNNG+1.1%NaCl | 15/20(75.0%)a (0.9±0.64) | 12/20(60%) | 4.5±4.4c | 0.7±0.7 | 6/20(30%) | 4.1±9.1 | 0.3±0.6 | Sarcoma |
| MNNG+MF | 10/19(53.0%) | 6/19(32%) | 1.2±2.3 | 0.7±1.1 | 3/19(16%) | 7.6±20.6 | 0.2±0.4 | Plasmacyto |

| | |
|---|---|
| (0.65±0.67) | ma |
| | Sarcoma |

(mean±SD)

*(    )    Number of tumor per rat

[a]: Significantly different from MNNG+MF value (P<0.05);

[b]: Significantly different from MNNG+10%miso value (P<0.05)

[c]: Significantly different from MNNG+MF value (P<0.01)

Table 3　Inhibition by 180-days fermented miso induction of gastric tumor's number and size by MNNG in CD rats[17]

| Group | Gastric tumor[1] | Adenocarcinoma[2] | |
| --- | --- | --- | --- |
| | | Number | Size (mm) |
| MF | 5.17±4.45 | 11 | 8.0±2.9 |
| 3-4 days fermented miso | 3.62±6.79 | 6 | 11.5±7.6 |
| 120 days fermented miso | 2.42±2.68 | 6 | 4.3±3.6[a] |
| 180 days fermented miso | 1.78±3.34[*] | 9 | 2.9±4.6[*,b] |
| NaCl | 3.23±4.08 | 8 | 5.1±4.1[a] |

(mean±SD)

[1.]　Included negative animal

[2.]　Positive animal values

[*]: Significantly different from MF value (P<0.05)

[a]: Significantly different from early-term value (P<0.05)

[b]: Significantly different from early-term value (P<0.01)

Table 4　Effects of dietary miso, NaCl and others on azoxymrthane (AOM)-induced aberrant crypt foci (ACF) in F344 rat colons[33-35]

| Group | ACF/colon | AC/colon | AC/focus |
|---|---|---|---|
| MF（Control） | 136.4±41.3 | 221.4±61.9 | 1.63±0.10 |
| 4.4% Sea salt | 39.8±22.0[**] | 62.9±38.1[**] | 1.69±0.48 |
| 4.4% Rock salt | 57.6±26.8[**] | 98.3±48.1[**] | 1.70±0.17 |
| 4.4% Home salt | 35.5±11.6[**] | 61.2±21.5[**] | 1.71±0.10 |
| 4.4% Coarse salt | 74.1±33.5[**] | 123.9±54.1[**] | 1.70±0.14 |
| 4.4% Pure NaCl | 100.4±62.9 | 159.8±95.6[*] | 1.61±0.23 |
| 2.2% Pure NaCl | 115.9±65.2 | 199.5±92.6 | 1.82±0.32 |
| 20% Miso | 66.3±41.1[**] | 124.4±78.0[**] | 1.88±0.18 |
| 10% Miso | 85.6±70.5[*] | 163.0±130.8 | 1.94±0.15 |
| 5% Miso | 134.1±52.2 | 240.9±92.9 | 1.83±0.17 |
| 3.0% kojic acid | 97.5±46.6[*] | 169.9±72.6 | 1.79±0.20 |
| 1.5% kojic acid | 91.6±31.3[*] | 167.2±53.4[*] | 1.84±0.20 |
| 5ppm Biochanin A | 131.0±63.0 | 226.6±90.0 | 1.81±0.27 |

（Maen±SD）

[*]: Significantly different from Control value (P<0.05)

[**]: Significantly different from Control value (P<0.01)

Table 5　Decrease by 180-day fermented miso of colon carcinoma's size induced by AOM in F344 Rats[47)]

| Group | Number of tumor | Size (mm) | Size of well-differentiated adenocarcinoma (mm) | Size of total tumor (mm) |
|---|---|---|---|---|
| 2 –year fermented miso+AOM | 0.50ear f | 4.60ear f | 9.72±5.43 | 11.40.6.37 |
| 180 days fermented miso+AOM | 0.85days | 2.72days [a] | 3.56±2.56[b] | 3.56days [a,**]9 |
| 3-4 days fermented　miso+AOM | 0.95days | 1.79days | 3.66±2.45[b] | 7.97days |
| MF+AOM | 1.10OMys | 3.76OMys | 6.86±3.38 | 7.26OMys |

(mean±SD)

a: Significantly different from 2 year fermented miso+AOM value (P<0.05)

b: Significantly different from 2 year fermented miso+AOM value (P<0.01)

Table 6　Inhibition by 180-days fermented miso of induction of pulmonary tumor
　by N-nitrosobis(2-hydroxypropyl)amine (BHP) in Wistar rats[73)]

| Group | No of animals | Adenoma | Adenocarcinoma | Tumor with PCNA-strongly positive |
|---|---|---|---|---|
| BHP | 8 | 62.6A-str | 6.56A-s | 21.0A-st |
| BHP+3-4 days fermented miso | 15 | 61.6±19.0 | 6.3±2.7 | 15.4±6.6[*] |
| BHP+180 days fermented miso | 14 | 57.8±24.9 | 2.9±1.7[**] | 11.7±4.7[**] |

(mean±SD)
[**]: Significantly different from BHP value (P<0.01)

Table 7　Blood pressure in male rats[92]

**Systolic blood pressure**

| Group | 0 week | 2 weeks | 4 weeks | 8 weeks | 12 weeks |
|---|---|---|---|---|---|
| Dahl Miso(2.3%) | | 124.2±10.3 | 144.0±13.0 | 158.8±20.8 | 158.8±19.4 |
| Dahl NaCl(2.3%) | | 131.4±11.4 | 147.8±9.6 | 181.0±15.0**, b | 191.6±13.4**, b |
| Dahl NaCl(1.9%) | | 134.2±10.1 | 151.6±9.6 | 181.2±19.2**, b | 189.8±30.2**, b |
| Dahl MF | 102.9±11.3 | 140.1±15.9 | 145.3±14.2 | 154.3±7.6 | 154.6±7.9 |
| SD Miso | | 117.6±15.8 | 130.0±8.7 | 135.5±16.1 | 132.5±15.0 |
| SD NaCl(2.3%) | | 127.4±14.2 | 141.0±17.7 | 142.8±12.0* | 136.6±7.2 |
| SD NaCl(1.9%) | | 117.0±13.8 | 144.6±12.5* | 132.0±16.6 | 129.4±6.5 |
| SD MF | 95.4±11.5 | 127.5±7.5 | 129.3±13.4 | 130.1±11.8 | 137.1±18.3 |

**Diastolic blood pressure**

| Group | 0 week | 2 weeks | 4 weeks | 8 weeks | 12 weeks |
|---|---|---|---|---|---|
| Dahl Miso(2.3%) | | 97.7±8.2 | 112.6±7.6 | 127.6±21.6 | 119.9±18.0 |
| Dahl NaCl(2.3%) | | 107.8±13.5 | 121.2±6.2**, a | 148.4±13.2*, a | 166.8±26.9**, b |
| Dahl NaCl(1.9%) | | 104.8±18.9 | 120.2±6.8a | 152.8±25.2*, a | 165.8±15.1**, b |
| Dahl MF | 77.5±11.6 | 108.3±9.8 | 113.8±13.5 | 127.9±7.8 | 123.9±8.4 |
| SD Miso | | 84.6±10.7 | 101.3±9.5 | 102.5±9.7 | 101.0±10.6 |
| SD NaCl(2.3%) | | 93.2±10.4 | 104.8±15.7 | 110.4±19.6 | 101.8±7.7 |
| SD NaCl(1.9%) | | 80.8±11.2 | 112.0±19.2 | 106.2±12.5 | 101.2±8.8 |
| SD MF | 68.7±8.5 | 93.6±9.5 | 97.1±13.8 | 102.4±7.5 | 112.2±14.1 |

*:　Significantly different from same strain MF (P<0.05), **: Significantly different from same strain MF (P<0.01)

a:　Significantly different from same strain Miso (P<0.05) b:　Significantly different from same strain Miso (P<0.01)

Table 8  Blood pressure in female rats[92)]

**Systolic blood pressure**

| Group | 0 week | 2 weeks | 4 weeks | 8 weeks | 12 weeks |
|---|---|---|---|---|---|
| Dahl Miso | | 121.9±10.8 | 134.4±11.4 | 145.0±12.9 | 143.1±16.0 |
| Dahl NaCl(2.3%) | | 118.4±8.4 | 153.0±5.6**, b | 161.4±7.6**, b | 173.6±15.8**, b |
| Dahl NaCl(1.9%) | | 114.0±10.0 | 141.4±14.5 | 142.8±19.2 | 146.6±8.0 |
| Dahl MF | 108.3±12.2 | 119.5±10.0 | 137.4±12.2 | 140.6±10.5 | 143.8±11.6 |
| SD Miso | | 114.9±11.7 | 133.5±7.0 | 131.7±12.9 | 132.2±12.1 |
| SD NaCl(2.3%) | | 116.0±14.4 | 139.4±9.1* | 136.8±10.9* | 142.4±7.9 a |
| SD NaCl(1.9%) | | 115.8±12.4 | 130.4±3.0 | 126.8±15.3 | 133.8±8.8 |
| SD MF | 95.9±11.5 | 115.7±8.6 | 124.8±12.2 | 124.2±10.9 | 132.0±16.8 |

**Diastolic blood pressure**

| Group | 0 week | 2 weeks | 4 weeks | 8 weeks | 12 weeks |
|---|---|---|---|---|---|
| Dahl Miso | | 93.8±5.7 | 107.7±12.7 | 112.3±10.5 | 110.9±23.1 |
| Dahl NaCl(2.3%) | | 97.2±10.4 | 116.0±13.8 | 123.0±8.8**, b | 141.8±7.3**, b |
| Dahl NaCl(1.9%) | | 82.8±21.2 | 116.2±12.2 | 111.4±11.9 | 120.4±6.9 |
| Dahl MF | 80.0±13.9 | 92.8±12.9 | 104.7±14.5 | 109.1±11.8 | 109.9±18.8 |
| SD Miso | | 85.9±8.5 | 108.4±7.5 | 99.2±12.6 | 99.4±5.5 |
| SD NaCl(2.3%) | | 88.2±4.8 | 106.6±11.1 | 114.4±11.3**, b | 111.6±13.0 |
| SD NaCl(1.9%) | | 86.6±13.6 | 101.4±6.1 | 95.0±10.4 | 104.2±14.2 |
| SD MF | 67.2±10.3 | 87.5±10.8 | 95.6±9.9 | 98.7±12.4 | 103.4±13.7 |

*:Significantly different from same strain MF (P<0.05); **:Significantly different from same strain MF (P<0.01)

a: Significantly different from same strain Miso (P<0.05); b: Significantly different from same strain Miso (P<0.01)