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Radioprotective Effects of Miso (Fermented Soy Bean Paste) Against Radiation in B6C3F1 Mice: Increased Small Intestinal Crypt Survival, Crypt Lengths and Prolongation of Average Time to Death

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ABSTRACT

The radioprotective effect of miso, a fermentation product from soy bean, was investigated with reference to the survival time, crypt survival and jejunum crypt length in male B6C3F1 mice. Miso at three different fermentation stages (early-, medium- and long-term fermented miso) was mixed in MF diet into biscuits at 10% and was administered from 1 week before irradiation. Animal survival in the long-term fermented miso group was significantly prolonged as compared with the short-term fermented miso and MF cases after 8 Gy of ^{60}Co-γ-ray irradiation at a dose rate of 2Gy min^{-1}. Delay in mortality was evident in all three miso groups, with significantly increased survival. At doses of 10 and 12 Gy X-irradiation at a dose rate of 4 Gy min^{-1}, the treatment with long-term fermented miso significantly increased crypt survival. Also the protective influence against irradiation in terms of crypt lengths in the long-term fermented miso group was significantly greater than in the short-term or medium-term fermented miso and MF diet groups. Thus, prolonged fermentation appears to be very important for protection against radiation effects.

Key words: *Radiation protection, Miso, B6C3F1 mouse*

With the JCO Company Ltd. accident two victims received bone marrow transplants and skin grafts but death due to gastrointestinal problems could not be prevented[6]. How to protect the gastrointestinal tract from irradiation is a very severe problem. Moreover, A-bomb survivors who had frequently consumed miso (Japanese soybean fermented paste) demonstrated decreased radiation damage[1]. Because of this report, Europeans were recommended to eat miso after the Chernobyl Accident. However, there have been few reports on animal experiments to confirm these beneficial effects. If gastrointestinal cell death were indeed ameliorated, miso would be very useful for prevention of radiation damage. The response of crypt stem cells to a variety of genotoxic and cytotoxic agents has been primarily studied using microcolony formation assays based on the capacity of surviving stem cells to regenerate cryptlike foci that can be scored histologically 3–4 days after irradiation[12-14]. Intestinal injury induced by ionizing radiation has been very extensively characterized. Cells in transit within the crypt cease replication after exposure to ionizing radiation, but continue to migrate out onto the villus. Thus, in the absence of surviving crypt stem cells, the crypts disappear. If one (or more) clonogenic stem cell survives irradiation, it will proliferate, ultimately giving rise to an entire regenerative crypt[12-14]. We have previously described the radiopreventative effects of miso against intestinal injury by X-irradiated mice[16]. To determine whether the soy beans themselves or the fermentation process might have a role, the present study was conducted to assess the effects of miso at various fermentation-stages on crypt survival after X-irradiation in mice.

MATERIALS AND METHODS

Animals

Six-week-old male B6C3F1 (Crj: B6C3F1) mice and our standard protocol for assessing radiation effects were used in the present experiment. The animals were housed in polycarbonate cages, five per cage, and kept under constant conditions of temperature (24 ± 2 °C) and humidity (50 ± 10%)

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with a 12 h light/12 h dark cycle. The mice were maintained according to the "Guide for Care and Use of Laboratory Animals" established by Hiroshima University and fed a commercial diet MF[9] (Oriental Yeast Co. Ltd., Tokyo, Japan) alone or with a 10% supplement of dried red miso, short-term fermented (immediate of fermentation), medium-term fermented (4 months of fermentation) or long-term fermented (6 months), from the Miso Central Institute, Tokyo, in biscuits. Normal tap water was also provided *ad libitum*.

Radiation

Groups of mice were whole body irradiated with 8 Gy of ^{60}Co-γ-ray (each 10 animals) at a dose rate of 2 Gy min^{-1} for the animal survival study and 7, 8, 10 or 12 Gy of X-rays for crypt survival (each 5 animals), delivered at a dose rate of 4 Gy min^{-1} as measured with a Radocon 555 dosimeter. The mice were not anaesthetized during the irradiation. Exposure factors were as follows: 200 kVp and a half-value layer 1.18 mm Cu. The X-ray air dose (in R) was then converted to the absorbed dose (in cGy) using a factor of 0.95 cGy/R.

One week before irradiation, the mice were given a diet supplemented with miso at different stages of fermentation and kept for 28 days on the same diet after ^{60}Co-γ-irradiation with 8 Gy. The animals were observed every day at 8:00, 12:00 and 18:00, and deaths were recorded for the animal survival experiment. In the other groups, the animals were kept for 3.5 days after irradiation then killed for determination of crypt survival.

Autopsy

Immediately after sacrifice, segments of the jejunum from the ileocecal junction (30 to 40 cm) were removed and fixed in Carnoy's solution. They were cut into several pieces, bundled together, embedded in paraffin, sectioned at a thickness of 3 μm and stained with hematoxylin-eosin. To quantitate the regenerating crypts, numbers of colonis per circumference were determined in cross-section[12-14]. In each mouse the number of surviving crypts in 10 gut cross-sections was scored, along with the crypt diameters of 20 longitudinally sectioned crypts.

Statistics

Statistical significance was determined with Dunnett's method and the Cox proportional hazard model for multiple comparisons using logarithmic transformation and the Student's *t*-test.

RESULTS

Ten days after the ^{60}Co-γ-irradiation, animals in the MF group started to die and all were dead after 19 days. A delay in mortality was evident in all three miso groups, with significantly increased survival in the short-term ($p<0.048$), medium-term ($p<0.026$) and long-term fermented miso groups ($p<0.011$) as compared with the MF group by the Cox model (Fig. 1).

The number of crypts in one circumference in the non-irradiated group was 123.7 ± 13.1 in the MF group and 125.2 ± 12.7 with aged miso. A dose dependent decrease was evident with 7-12 Gy (see Table 1). After 7 Gy, irradiation was significantly greater with medium-term and short-term fermented miso as compared to the MF group. With 8 Gy, surviving crypts in the short-term and long-term fermented miso groups were also significantly increased over the MF value. Crypt survival was evident with a significant difference in the long-term fermented miso group ($p < 0.01$) as com-

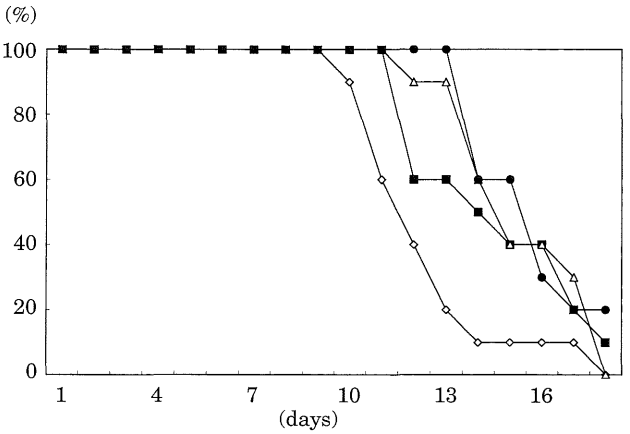

Fig. 1. Animal survival after ^{60}Co-γ-ray irradiation with 8 Gy
◇ – ◇: MF diet
● – ●: Long-term fermented miso
△ – △: Medium-term fermented miso
■ – ■: Short-term fermented miso

Table 1. Numbers of surviving crypts after X-irradiation

	0 Gy	7 Gy	8 Gy	10 Gy	12 Gy
Long-term fermented miso	125.2 ± 12.7	103.0 ± 11.6	87.4 ± 9.1**	68.5 ± 9.3**	50.0 ± 5.2**
Medium-term fermented miso		108.4 ± 13.9**	84.4 ± 11.7	55.1 ± 5.4**,a	43.9 ± 5.1**,a
Short-term fermented miso		112.5 ± 14.0**	97.3 ± 13.7**	53.0 ± 6.4*,a	41.0 ± 6.5**,a
MF	123.7 ± 13.1	97.6 ± 10.8	80.8 ± 8.9	48.7 ± 7.1	30.3 ± 5.8

(mean ± SD)
**: Significantly different from MF ($p<0.01$) by Dunnett's method
[a]: Significantly different from Long-term fermented miso ($p<0.01$)

Table 2. Effects of irradiation on jejunum crypt lengths (μm)

	0 Gy	7 Gy	8 Gy	10 Gy	12 Gy
Long-term fermented miso	169.1 ± 24.0	241.1 ± 27.2**	204.5 ± 28.8	149.8 ± 27.4**	83.3 ± 33.4
Medium-term fermented miso		235.8 ± 36.5**	182.5 ± 23.5**,a	148.7 ± 23.4**	86.4 ± 26.6
Short-term fermented miso		224.4 ± 28.0[a]	190.4 ± 24.5[a]	123.8 ± 30.9[a]	75.5 ± 24.6
MF	160.3 ± 20.8	224.3 ± 28.0	198.3 ± 32.7	132.7 ± 29.9	82.2 ± 25.4

(mean ± SD)
**: Significantly different from MF ($p<0.01$) by Dunnett's method
[a]: Significantly different from Long-term fermented miso ($p<0.01$)

pared with the medium-term fermented miso and MF groups by the Cox model. The lengths of surviving crypts with 7 and 8 Gy irradiation were increased as compared with those at 0 Gy. With 10 and 12 Gy they were significantly decreased. Ameliorating effects were observed in all miso groups (Table 2). Crypt lengths in the long- and medium-term fermented miso groups after 7 and 10 Gy irradiation were significantly increased as compared to those in the MF group (Table 2).

DISCUSSION

The present paper documents a significant increase in the survival of crypts and crypt lengths in animals receiving fermented miso associated with a prolongation of average time to death after ^{60}Co-γ-irradiation. Previously we reported that miso[16] and soy sauce[17] increased crypt survival for radiation injury, and this is also the case for Caucasus region yogurt (Watanabe et al, unpublished data). No protective effects were evident when miso was given after X-irradiation[16] so that we can conclude that fermented substances protect not only against gastrointestinal damage but also bone marrow death due to radiation. Recently, Houchen et al[7] reported that expression of FGF-2 is induced with radiation injury and recombinant human FGF-2 markedly enhanced crypt survival. Takahama et al[15] 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[3] have reported that recombinant human keratinocyte growth factor can protect mice from chemotherapy- and radiation-induced gastrointestinal injury and mortality but not whole-body radiation, at least in terms of death from intestinal and marrow toxicity. We also found VEGF to have a protective influence (Katoh and Watanabe, unpublished data). Cytokine-like substances in miso may thus play an important role in the protection 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. Further study is needed to elucidate 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.

There have been a number of reports of protection by miso against carcinogenesis in different organs. Incidences of spontaneous, radiation and chemical induced liver tumors, were, for example, found to be decreased[8,10]. Mammary tumor induction was also reduced[2,4,5], and our group earlier described gastric tumors to be decreased by miso induced by N-methyl-N'-nitro-N-nitrosoguanidine[18]. Masaoka et al[9] reported that colon aberrant crypt foci induced by azoxymethane were similarly reduced by miso in dose dependence. Recently, we found that this is particularly the case with long-term fermented miso[11]. Thus, the radiation protection and tumor prevention effects of miso might be due to fermentation. However, to our knowledge there are no reports regarding the effective substances in miso after different periods of fermentation. What other responsible components might be and specifically those which increase with the period of fermentation, remains to be determined.

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