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

Masayuki OHARA, Huimei LU, Katsutomo SHIRAKI, Yoshimasa ISHIMURA,
Toshihiro UESAKA, Osamu KATO and Hiromitsu WATANABE*

Department of Environment and Mutation, Research Institute for Radiation Biology and Medicine,
Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima, 734-8553, Japan

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 irra-
diation. 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 60Co-γ-ray irradiation
at a dose rate of 2Gy min⁻¹. Delay in mortality was evident in all three miso groups, with signifi-
cantly 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 vic-
tims received bone marrow transplants and skin
grafts but death due to gastrointestinal problems
could not be prevented[6]. How to protect the gas-
trointestinal tract from irradiation is a very severe
problem. Moreover, A-bomb survivors who had fre-
quently consumed miso (Japanese soybean fer-
mented 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 preven-
tion of radiation damage. The response of crypt
stem cells to a variety of genotoxic and cytotoxic
agents has been primarily studied using micro-
colony 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 ioniz-
ing radiation has been very extensively character-
ized. 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, ulti-
mately giving rise to an entire regenerative
crypt[12-14]. We have previously described the radi-
preventative effects of miso against intestinal
injury by X-irradiated mice[16]. To determine
whether the soy beans themselves or the fermen-
tation process might have a role, the present study
was conducted to assess the effects of miso at vari-
ous 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%).
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 (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 \(^{60}\text{Co}-\gamma\)-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 \(^{60}\text{Co}-\gamma\)-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 \(\mu\)m and stained with hematoxylin-eosin. To quantify the regenerating crypts, numbers of colonies per circumference were determined in cross-section.  

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 \(^{60}\text{Co}-\gamma\)-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 \(^{60}\text{Co}-\gamma\)-ray irradiation with 8 Gy](image)

![Table 1. Numbers of surviving crypts after X-irradiation](table)

(mean ± SD)  
**: Significantly different from MF (\(p<0.01\)) by Dunnett’s method  
*: 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 60Co-γ-irradiation. Previously we reported that miso and soy sauce 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 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. reported that expression of FGF-2 is induced with radiation injury and recombinant human FGF-2 markedly enhanced crypt survival. Takahama et al. 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. 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 increase with the period of fermentation, remain for a significant time 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. Mammary tumor induction was also reduced, and our group earlier described gastric tumors to be decreased by miso induced by N-methyl-N-nitro-N-nitrosoguanidine. Masaoka et al. 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. 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.

**ACKNOWLEDGEMENTS**

We thank Dr. Malcolm Moore, Asian Pacific Organization for Cancer Prevention, Director APOCP Training Center, for his reading of this paper and Mr. T. Nishioka and Ms H. Hamada for their technical assistance.

(Received September 7, 2001)  
(Accepted October 11, 2001)

**REFERENCES**


---

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 Gy</th>
<th>7 Gy</th>
<th>8 Gy</th>
<th>10 Gy</th>
<th>12 Gy</th>
<th>16 Gy</th>
<th>20 Gy</th>
<th>24 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term fermented miso</td>
<td>169.1 ± 24.0</td>
<td>241.1 ± 27.2**</td>
<td>204.5 ± 28.8</td>
<td>149.8 ± 27.4**</td>
<td>83.3 ± 33.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium-term fermented miso</td>
<td>160.3 ± 20.8</td>
<td>224.4 ± 28.0*</td>
<td>190.4 ± 24.5*</td>
<td>123.8 ± 30.9*</td>
<td>75.5 ± 24.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-term fermented miso</td>
<td>235.8 ± 36.5**</td>
<td>182.5 ± 23.4**</td>
<td>148.7 ± 23.4**</td>
<td>86.4 ± 26.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF</td>
<td>190.4 ± 28.0</td>
<td>149.8 ± 27.4**</td>
<td>83.3 ± 33.4</td>
<td>75.5 ± 24.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(mean ± SD)

**:** Significantly different from MF (p<0.01) by Dunnett’s method  
*: Significantly different from Long-term fermented miso (p<0.01)
factor protects mice from chemotherapy and radiati-
nitroso-N-methylurea-induced rat mammary carci-
ogenesis by soy foods or biochanin A. Jpn. J. Cancer Res. 89: 137–142.
nitroso-N-methylurea rat mammary cancer by miso and 
dose estimation for 3 severely exposed patients in the 
stem cell survival and its expression is induced after radiation injury. Am. J. Physiol. 276: 
G249–G258.
development of colonic aberrant crypt foci induced by azoxymethane in F344 rats. Nut. Cancer 32: 
testosterone on liver tumors induced by a combined treat-
Prevention by long-term fermented miso of induced of 
colonic aberrant crypt foci by azoxymethane in 
colony assay in mouse small intestine, p. 50–60. In C.S. Potten and J.H. Hendry (eds.), Cell Clones: 
Manual of Mammalian Cell Techniques. Churchill 
Livingstone, Edinburgh.
Pretreatment with transforming growth factor β-
protects small intestinal stem cells against radia-
16. Watanabe, H., Takahashi, T., Ishimoto, T. and 
Ito, A. 1991. The effect on miso diet of small intesti-
nal damage in mice irradiated by X-rays. Miso 
suppression and radiation protection of miso diets 
(in Japanese)
18. Watanabe, H., Uesaka, T., Kido, S., Ishimura, 
concomitant Miso or NaCl treatment on induction 
of gastric tumors by N-methyl-N-nitro-N-