

Radioprotection and anti-cancer effect of Hatakeshimeji (*Lyophyllum decastes*)

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Abstract

Our preceding studies have demonstrated that Hatakeshimeji (*Lyophyllum decastes*) extracts have some radiation-protection and anti-tumor effects, and, as for the mechanism of the action, the enhancement of immuno activity and the action of anti-oxidation have been elucidated. In this study, we examined anti-tumor effects, the protection of immuno disturbance and the anti-oxidation action, against radiation in Hatakeshimeji. After an intra-abdominal inoculation of approximately 2×10^6 Sarcoma 180, ICR mice were given an endoceliac dosage 200 mg/kg of Hatakeshimeji every other day for two weeks. Two Gy of radiation was given three times and the numbers of leukocytes and lymphocyte were counted. We also measured body weight and tumor size two weeks after seeding the cancer cells. Anti-oxidation action was measured using the AAPH (2,2-azobis (2-amidinopropane) dihydrochloride) method. Comparing with that the group inoculated only with cancer cells increased the tumor size with time, some decrease in tumor size was clearly demonstrated in the group of radiation exposure

groups and administration of Hatakeshimeji group. Almost all the mice inoculated only with cancer cells died after two weeks of radiation, while two thirds of radiation and Hatakeshimeji groups lived. The leukocytes, the lymphocyte counts, increased when doses Hatakeshimeji were administered regardless of radiation exposure. Anti-oxidation action was also demonstrated in both groups of Hatakeshimeji. These results may indicate that administration of Hatakeshimeji enhances the immunologic activity and prevent side effects during cancer radiotherapy and provides a supplemental tool for the cure of cancer.

Introduction

Hatakeshimeji is an edible mushroom, belonging to the *Lyophyllum* genus, and named *Lyophyllum decastes* Sing. It particularly resembles *L. shimeji* Hongo in its taste and touch. It is a stump mushroom, which grows in forest, having a grayish brown-shaped umbrella about 4–9 cm in diameter¹⁾. Hatakeshimeji (*Lyophyllum decastes* Sing) was called the *Lyophyllum* aggregate and has highly been valued for a long time, and its artificial cultivation was difficult till now. Recently, there has been artificial cultivation using bacterial strains as seedlings [Kameyama No1]²⁾. Now we can commonly see it at a general retail store. Authors have studied Hatakeshimeji for its anti-tumor, angiotensin converting enzyme-inhibiting and serum cholesterol-decreasing activities^{2,3)}. We have found that Hatakeshimeji has a greater activity than the same class of shiitake regarding anti-tumor activity and made clear that the acid β -1,6 and β -1,3 D-glucans, and their active bodies β -1,6 and β -1,3 D-glucans are contained in Hatakeshimeji⁴⁾. We report here the effects of Hatakeshimeji extracts in radiotherapy of cancer bearing mice.

Materials and Methods

Hatakeshimeji extract

A hot water extract was obtained from 500 kg of Hatakeshimeji fruit body using 5 kl of boiling distilled water and a heating duration of one hour, and its aerosol-dried material was used for the experiments.

Mouse

We obtained ICR/Slc corollary mice (five-week instars, males) from Japanese SLC Co., Ltd. After receiving, the mice were reserve-bred for one week

and only healthy mice were used for the examinations. The mice were freely fed on commercial chow (CA-1, Japan CLEA Co., Ltd.) and water and bred under a light cycle of 12 hours (8:00 lighting, 20:00 lights out). Air humidity and temperature of the room were 60–65% and $22 \pm 2^\circ\text{C}$, respectively.

Animal experiment

We set the following experimental groups: control group (C), tumor seeding group (T), tumor seeding + X-ray exposure group (TX), Hatakeshimeji administrated group (H), Hatakeshimeji + tumor seeding group (HT), Hatakeshimeji + tumor seeding + X-ray exposure group (HTX).

X-ray exposure

We used an X-ray generator (Philips MG226/4.5). As for the pipe voltage, 200 kV dose was irradiated at the rate of 0.35 Gy/min. The total quantity of exposed X-ray was 2 Gy. The exposure place of mice was fixed with a holder in the position of left foot front (the place that was seeded cancer cells), and the places that were not irradiated were covered with a lead container. We irradiated X-ray on the 19th day and the 22nd day.

Tumor seeding

Tumors were seeded on the 15th day after breeding the mice for one month or more. We inoculated about 2×10^6 Sarcoma 180 cancer cells in the left foot muscle of ICR male mice.

Administration of Hatakeshimeji extract

The extract was dissolved in a saline solution. For the H, HT and HTS groups, an endoceliac dosage of hot water educts (100 mg/kg) was administered every other day for weeks. For the C, T, and TX

groups, only saline was injected. We measured tumor size every week after two weeks of the cancer-cell seeding. We also measured body weight in a schedule just the same as the measurement of tumor size.

The each group consisted of six mice. The tumor size was measured using the following formula :

$$\text{Tumor size (cm}^3\text{)} = \frac{3}{4} \pi \frac{A^2 B}{2}$$

A ; Minor axis (cm)

B ; Longer axis (cm)

At 14 (just before the first X-ray exposure), 16, 20, 23, 25, 29, and 37 days after the tumor seeding, each mouse was fixed with a holder, and it's caudal vein with a spits scalpel to an extend for obtaining approximately 20 ul of dripping blood. The blood was collected in a blood-collecting vessel (Dolamond company), and diluted with a diluent (Nihon Kohden). White blood cell counts including lymphocyte counts and granulocytes counts were measured using an automated blood cell analyzer (Nihon Kohden, Celltac α MEK-6318). The results obtained were expressed in a mean \pm standard deviation (S.D.). The student's t-test was applied and results over 0.05

were considered to be significant.

Results

Number of implantations

Change in the number of peripheral blood cells Leukocytes

Time-dependent changes in leukocyte number in each group by *Lyophyllum* administration is shown in Fig. 1.

The *Lyophyllum* group showed a significant increase in the leukocyte cell number, as compared with the control group. The ratio, significantly increased throughout the whole period of treatment, was 5/8 (*Lyophyllum* group). These results suggest that *Lyophyllum* definitively increase the number of leukocytes.

In the irradiation groups, the number of leukocytes began to decrease in all the experimental groups just after irradiation, and then there was a tendency to recover after 3 days. When compared with the X group over the whole period, the number

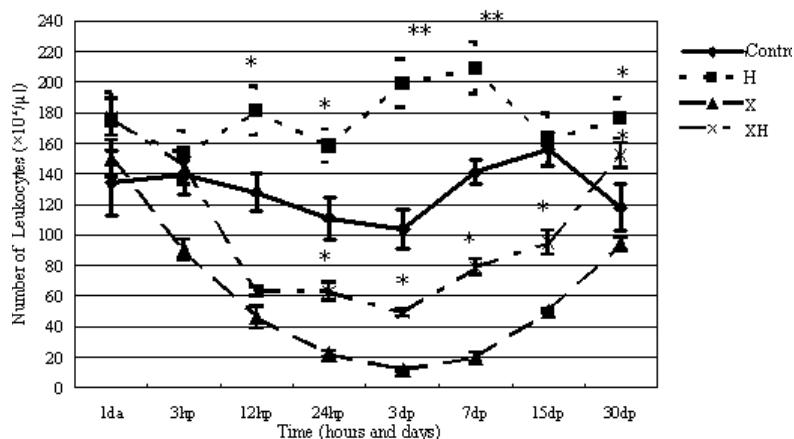


Fig. 1. The change in the number of leukocytes in the blood taken from the tail vein of whole body irradiated mice. Water extract *Lyophyllum* (250mg/kg) was given i.p. at 2 weeks before irradiation. Each line-graph represents the mean value \pm SE for 10 male mice leukocytes. * ; P<0.05, ** ; P<0.01

of leukocyte was always higher in the LX group. When observed at each period, the number of leukocytes in the LX group was significantly higher than in the X-ray alone group the day before irradiation ($P < 0.05$). This suggests that the increase in the number of leukocytes is shown in a non-irradiated state. Significant ratios were observed in the LX group (6/8). These results suggest a reduction of the X-ray-induced decrease in leukocyte number in the LX group.

Lymphocytes

Time-dependent changes in the number of lymphocytes by *Lyophyllum* administration in each non-irradiated and 2 Gy systemic irradiation group is shown in Fig. 2.

In the non-irradiation groups, all the treatment groups showed a high lymphocyte number as compared with the control group. Specifically, there was a significant ratio in the *Lyophyllum* group (4/8), statistically significant differences were observed in many periods ($P < 0.05$). Overall, there was a ten-

dency for lymphocytes to increase, as compared with the control group.

This is the same tendency as seen in the non-irradiation groups. With respect to time-dependent change after irradiation, all the experimental groups showed a decrease and subsequent restoration was observed 3 days after irradiation. The LX group also showed higher lymphocyte numbers than the X group. A significant ratio was observed in the LX group (4/8) ($P < 0.05$). These treatments were shown to be especially effective in the restoration period 3 days after irradiation.

Antioxidant activity (AOA_{AAPH})

No effect of any treatment was demonstrated. From these results, it was confirmed that there was no antioxidant activity against peroxyradical following any treatment.

Effect on tumors

Measurement of the rate of tumor growth by *Lyophyllum* administration is shown in Fig. 3. The

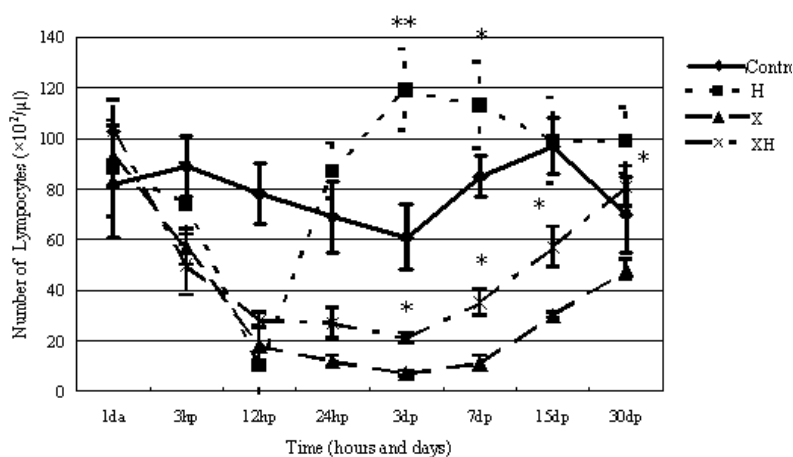


Fig. 2. The change in the number of lymphocytes in the blood taken from the tail vein of whole body irradiated mice. Water extract *Lyophyllum* (250 mg/kg) was given *i.p.* at 2 weeks before irradiation. Each linegram represents the mean value \pm SE for 10 male mice leukocytes. * ; $P < 0.05$, ** ; $P < 0.01$

number of days required for a doubling of the tumor size and the ratio between the control group and the X group.

Doubling times in the non-irradiation group were not different from those in the control group. In the topical radiation groups (therapy group), doubling times in the LX group were 1.4-fold longer, as compared with the control group. Doubling times in the LX group did not exceed those in the X group and tended to be slightly shorter. It was confirmed that tumor growth was suppressed, as compared with the X group.

Discussion

White blood cells, which consist of lymphocytes, granulocytes and monocytes, are deeply involved in immunity. Lymphocytes are classified roughly into T-cells and B-cells. T-cells are associated with cellular immunity, whereas B-cells are associated with humoral immunity through the production of antibodies. T-cells are further classified into helper T- and suppresser T-cells, Helper T cells play a role in the in-

struction and activation of B cells, NK cells, killer T cells and cytotoxic T cells. Granulocytes migrate to the peripheral tissues through the blood vessel walls, and phagocytose bacteria and foreign bodies. Monocytes are converted into macrophages by undergoing morphological changes after migration to the tissues, and transmit antigen information to T lymphocytes. Furthermore, macrophages activate NK cells and LAK cells. Thus, among white blood cells, lymphocytes give instruction by distinguishing self and non-self, and play a central role in the immune reaction⁵⁾. In this study, in the non-irradiation groups, the *Lyophyllum* group showed an increase of the number lymphocytes, granulocytes and monocytes, and an increase in the overall white blood cell number was suggested.

Lyophyllum is a mushroom which contains abundant β - (1-3) -D-glucan, and β - (1-6) -D-glucan. Petruczenko et al. have reported an increase in the number of granulocytes in response to β - (1-3) - D-glucan⁶⁾. Treatment with β - (1-6) -D- glucan has also been reported to cause activation of macrophages⁷⁾,

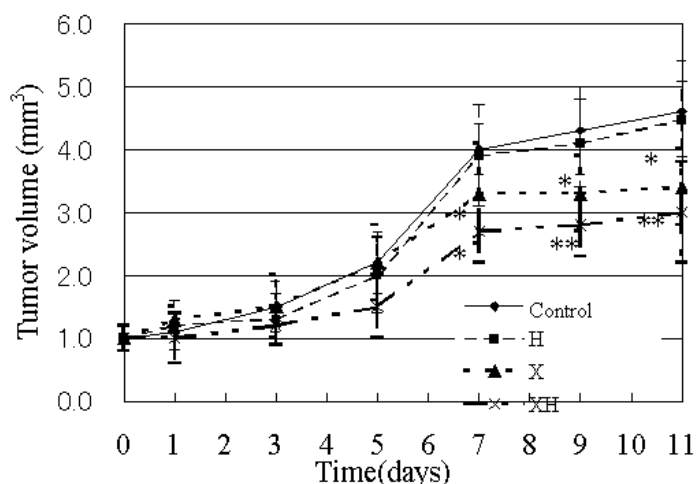


Fig. 3. Antitumor effect by the *Lyophyllum* (*Hatakeshimeji*) medication on C3H mice. Each line represents mean value \pm S.E. Analyzed by Dunnett-test. * ; $P < 0.05$, ** ; $P < 0.01$ vs C group.

increase of T-cells⁸⁾ (Mizuno et al.), and increase in release of TNF- α and IL-8 through activation of macrophages⁹⁾. However, it has also been reported that intraperitoneal administration of these polysaccharides induce inflammation in the peritoneal cavity due to difficulty of intestinal absorption¹⁰⁾. Furthermore, it is reported that β -(1-6)-D-glucan accumulates in liver and spleen after peritoneal and oral administration¹¹⁾.

The present study and previous reports strongly suggest the involvement of β -(1-3)-D-glucan and β -(1-6)-D-glucan. It is possible that macromolecular polysaccharides such as β -(1-3)-D-glucan stimulate intestinal immunity due to a slight inflammatory state induced by the difficulty of absorbing these substances in the peritoneal cavity and intestine. Intestinal intraepithelial T lymphocytes exist among the intestinal mucosal epithelial cells, and Peyer patches and lymphoid tissue are found in the periphery of the digestive tract. Since 70-80 % of B lymphocytes exist in the intestinal lymphoid tissue, the intestine is regarded as the greatest immune tissue¹⁴⁾. However, except for β -(1-3)-D-glucan, there are few reports that food is directly associated with the immune system, regardless of how difficult the absorption may be.

Therefore, it is thought that this results from some subtle difference in physico-chemical structure, such as the side chains of β -(1-3)-D-glucan. In addition, it is necessary to consider the relationship with enterobacteria, because β -(1-6)-D-glucan is degraded by beneficial enterobacteria and relatively well absorbed¹⁵⁾. Thus, it is speculated that macrophages are activated by the intestinal immune system, IL-8 and TNF- α are released from macrophages, helper T cells are activated, and the systemic immune system, consisting of macrophages, cytotoxic T cells, killer T cells, NK cells and B cells, is activated.

However, there are no sufficient grounds to connect these series of mechanisms, and further experiments are required.

Injury of cells is the most important side effect of radiation, and the lymphocyte is the most sensitive cell¹²⁾, with radiation causing interphase death in the short term¹³⁾. Thus, control of radiation effects on lymphocytes, which are integral to the immune system, is extremely important, and effects on lymphocytes can be regarded as an index of radiation-induced injury of cells. When radiation is applied to the body, free radicals such as H \bullet , OH \bullet and O $_2^-$ (superoxide anion) are generated by radiation-induced degradation of water molecules. Free radical-induced injury of DNA is called indirect action¹⁴⁾. Freeman et al. reported that administration of oxidation-reduction agents reduced cell injury induced by O $_2^-$ generated by ionizing radiation¹⁵⁾.

It is suggested that the administration of *Lyophyllum*, which shows a radioprotective effect, does not inhibit the radiation-induced suppression of tumor growth, but suppresses tumor growth independently. This could be because blood flow is poor in cells containing low oxygen, such as tumor cells¹⁶⁾, and there are less radical scavenger factors in the plasma. Thus, the antitumor constituents and tumor suppressive factors in these substances would act by activation of immune cells.

In this study, we examined tumor growth of only Sarcoma 180, and it is necessary to explore various kinds of tumors, and to employ more accurate experimental systems in the future.

References

- 1) Ukawa Y, Ito H, Hisamatsu M: Antitumor effects of (1 \rightarrow 3)- β -D-glucan and (1 \rightarrow 6)- β -D-glucan purified from newly cultivated mushroom, Hatakeshimeji (*Lyophyllum decastes* Sing.). J.

- Biosci. and Bioeng. 90, 98-104, 2000.
- 2) Ukawa Y, Andou M, Nishii T: Angiotensin I-converting enzyme inhibitory activity and antitumor activity of Hatakeshimeji (*Lyophyllum decastes* Sing.). Nippon Shokuhin Kagaku Kogaku Kaishi 48, 58-63, 2001.
 - 3) Ukawa Y, Andou M: Hypocholesterolemic activity of Hatakeshimeji (*Lyophyllum decastes* Sing.) mushroom in rats. J. Jpn. Soc. Food Sci. Tech. 48, 22-26, 2001.
 - 4) Cleland CL: Integrating recent advances in neuroscience into undergraduate neuroscience and physiology courses. Adv. Physiol. Educ. 26, 271-277, 2002.
 - 5) Cassone A, Bistoni F, Cenci E, et al.: Immunopotential of anticancer chemotherapy by *Candida albicans*, other yeasts and insoluble glucan in an experimental lymphoma model. Sabouraudia 20, 115-125, 1982.
 - 6) Petruczenko A: Glucan effect on the survival of mice after radiation exposure. Acta Physiol. Pol. May-Jun 35, 231-236, 1984.
 - 7) Sakurai T, Hashimoto K, Suzuki I, et al.: Enhancement of murine alveolar macrophage functions by orally administered beta-glucan. Int. J. Immunopharmacol. 14, 821-830, 1992.
 - 8) Mizuno M, Morimoto M, Minato K, et al.: Polysaccharides from *Agaricus blazei* stimulate lymphocyte T-cell subsets in mice. Biosci. Biotechnol. Biochem. ; 62, 434-437, 1998.
 - 9) Sorimachi K, Akimoto K, Ikehara Y, et al.: Secretion of TNF-alpha IL-8 and nitric oxide by macrophages activated with *Agaricus blazei* Murill fractions in vitro. Cell. Struct. Funct. 26, 103-108, 2001.
 - 10) Takaku T: Antitumor action of *Agaricus blazei* Murill and its adverse reactions. Ehime. Medicine 19, 523-533, 2000.
 - 11) Fujiyama Y, Suzuki Y, Oshiman K: Selective tumoricidal effect of soluble proteoglycan extracted from the basidiomycete, *Agaricus blazei* Murill, mediated via natural killer cell activation and apoptosis. Cancer Immunol. Immunother. 46, 147-159, 1998.
 - 12) Gu YH, Kai M, Kusama T: The embryonic and fetal effects in ICR mice irradiated in the various stages of the preimplantation period. Radiation Research. 147, 735-740, 1997.
 - 13) Gu YH, Hasegawa T, Suzuki I, et al.: A study of the radioprotection effect of guarana (*Paullinia cupana*) on the fetuses of ICR mice. J. Kor. Radiat. Prot. 26, 347-356, 2001.
 - 14) Gu YH, Suzuki I, Hasegawa T, et al.: Malformation effects in ddY mice irradiated at two stages in the preimplantation period. J. Jpn. Radio. Soc. 62, 92-97, 2002.
 - 15) Mishima S, Saito K, Gu YH: Antioxidant and immuno-enhancing effects of *Echinacea purpurea*. Biol. Pharm. Bull. 27, 1004-1009, 2004.
 - 16) Miyata K, Hasegawa T, Gu YH, et al.: The activation of immunological activity and anti-tumor effects by mild hyperthermia. Jpn. J. Hyperthermic Oncol. 21, 33-45, 2005.