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Radiomitigative Effects of *Grifola frondosa* Preparations on Mice Exposed to Lethal Ionizing Irradiation

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Abstract

Maitake mushroom *Grifola frondosa* (*GF*) is a polyporaceous fungus, increasingly recognized as a good source of polysaccharide compounds with dramatic health-promoting potential. To examine the radiomitigative effect of *GF*, we tested the effect of seven preparations of this fungus on the survival of mice exposed to lethal ionizing radiation. We tested freeze-dried fungal body of *GF* (YM-6A), α-glucans obtained from YM-6A (YM-2A), fiber components of *GF* (YM-11), *GF* mycelium (YM-51), hot-air dried ingredients (MD), 1:1 mixture of MD and YM-6A (YM-52), and crystal ergothioneine (YM-44). Each preparation was administered orally to 8-week-old mice for 7 consecutive days at 10 mg/kg/day before X-irradiation. All non-treated individuals died by day 16. The survival rate among the mice treated with YM-6A was 20% on day 30. One YM-52-treated mouse also survived until day 28. However, other preparations (YM-2A, YM-11, YM-44, and YM-51) had no radiomitigative effect. None of the substances used in the study affected the growth of non-irradiated control mice, demonstrating that these preparations were not toxic. We conclude that *GF* can be ingested with daily food and some of the preparations can counteract the harmful effects of radiation exposure, such as low-dose, long-term exposure.

Introduction

Maitake mushroom, *Grifola frondosa* (*GF*), is a polyporaceous fungus, increasingly recognized as an excellent source of polysaccharide compounds with many health benefits [1]. *GF* extracts have shown particular promise as immunomodulating agents and as adjuncts in cancer and HIV therapies. They may also provide some benefit in the treatment of hyperlipidemia, hypertension, and hepatitis. Ma et al. have suggested that *GF* increases glucose metabolism and synthesis of intracellular glycogen through the Akt/GSK-3 pathway. They have concluded that *GF* can be considered a potential source of the molecules with antidiabetic properties [2].

Ionizing radiation with low-linear energy transfer, such as X-rays, produces reactive oxygen species by indirectly affecting the water molecules. Such irradiation also generates potentially lethal double-strand DNA breaks, triggering apoptosis or stress-related responses. Exposure to a lethal dose of ionizing radiation causes severe acute radiation syndrome (ARS) involving bone marrow and gastrointestinal tissue death. ARS is associated with a decrease in the peripheral blood cell count and gastrointestinal dysfunction, ultimately leading to death due to systemic bleeding [3]. To alleviate these effects, reconstitution and restoration of hematopoiesis is a top priority. Some previous studies regarding physiological activities of *GF* have shown that the polysaccharides obtained from *GF* promote hematopoiesis

in vitro and *in vivo* [4–9]. β-glucans derived from *GF* induce the production of granulocyte colony-stimulating factor (G-CSF), one of the hematopoietic cytokines, and stimulate hematopoietic progenitor cells, giving rise to white blood progenitor cells [6, 7]. Therefore, *GF* is expected to have radioprotective/radiomitigative effects; however, these properties have not been extensively studied.

To examine the radiomitigative properties of *GF*, we tested the effect of seven different *GF* preparations on the survival of mice exposed to lethal ionizing radiation.

Materials and Methods

Mice

Female C57BL/6J Jcl mice were delivered at 7 weeks of age from the breeding facilities of Clea Japan (Tokyo, Japan) and housed in a conventional animal room with a 12-h light/dark cycle. The mice received food and water ad libitum. All experiments were conducted according to the legal regulations and the Guidelines for Animal Experiments of Hirosaki University. The mice were divided into seven groups, each of which was administered a different preparation of *GF* for 7 consecutive days before a lethal dose of X-irradiation. The control mice received the same *GF* ingredients, but they were not irradiated.

Treatment with seven *GF*-derived preparations

The seven *GF* preparations were provided by Yukiguni Maitake Co., Ltd. (Nigata, Japan). These were the freeze-dried fungal body of *GF* (YM-6A), α-glucan obtained from YM-6A (YM-2A), fiber components of *GF* (YM-11), *GF* mycelium (YM-51), hot-air dried ingredients (MD), 1:1 mixture of MD and YM-6A (YM-52), and crystal ergothioneine (YM-44). Briefly, each fraction was prepared as follows; YM-6A: the *GF* was stored for 3 days at 10 ºC before harvest, and fruit bodies thus obtained were lyophilized. The lyophilized material was considered to be powder. The powder was extracted with distilled water for 30 min at 121 ºC, then the filtrate was concentrated. YM-51: broth and mycelium of *GF* was extracted by using autoclave for 15 min at 100 ºC and then extraction solution was centrifuged at 7000 rpm for 30 min. The supernatant was treated with 75% ethanol and the precipitate was lyophilized. MD: the hot-air dried *GF* fruiting body powder was treated for 30 min at 121 ºC in distilled water, then ethanol was added to the concentrated extraction until as to be final volume concentration of 50%. After removing insoluble matter, the extraction was concentrated. Each preparation was orally administered to 8-week-old mice for 7 consecutive days at 10 mg/kg/day.

Exposure to X-irradiation

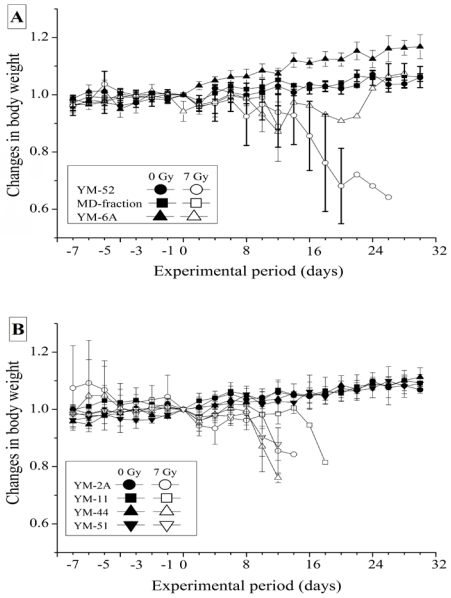
The mice were exposed to 7 Gy total body irradiation at a rate of 1.0 Gy/min, using Hitachi MBR-1520R-3 device with settings of 150 kV and 20 mA (Hitachi, Tokyo, Japan). The beam was filtered through 0.5-mm aluminum and 0.3-mm copper plates. The dose of X-irradiation was determined based on the 30-day survival obtained in various pilot experiments. We decided to expose the mice to a 7-Gy dose of X-irradiation, which resulted in a 0% 30-day survival rate.

Results

Changes in body weight

To evaluate the effects of each *GF* fraction on the individual growth, the mice were weighed during the experimental period (Figure 1). The preparations were administered orally for 7 consecutive days before X-irradiation. The treated mice and control mice showed similar growth curves during this period, indicating that the administration of *GF* did not have toxic effects (filled symbols in Fig. 1A and B). However, the body weight of irradiated mice decreased in all cases. The weight of surviving mice treated with YM-6A recovered 18 days after irradiation.

Figure 1. Survival trends after X-ray irradiation of the mice pre-treated with *GF*-products. The survival rate was calculated using the Kaplan–Meier method. [A], YM-6A, YM-52, and MD administration. [B], YM-2A, YM-11, YM-44, and YM-51 treatment. The data for mice not treated with *GF* products (control, marked with "7 Gy" only) are also shown.

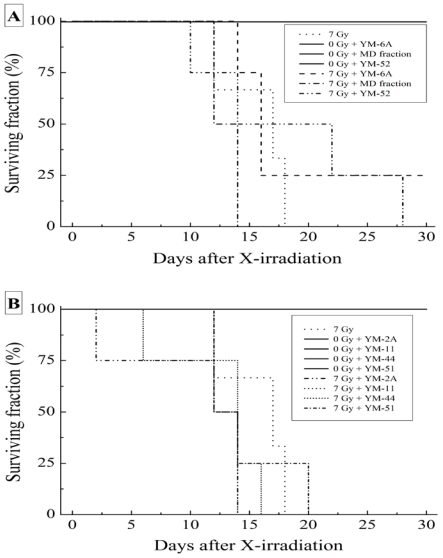


Survival rates of mice exposed to lethal X-irradiation

The radiomitigative effects of each *GF* preparation on the survival of mice exposed to a lethal 7-Gy dose of X-irradiation were tested. As shown in Figure 2, the YM-6A treatment resulted in 20% survival of irradiated individuals on day 30. Most

other preparations (YM-2A, YM-11, YM-44, and YM-51) had no radiomitigative effect, and only one YM-52-treated mouse survived until day 28 (Fig. 2A).

Figure 2. Weight changes for the irradiated (filled symbols) and not-irradiated (empty symbols) mice pre-treated with various *GF* products.
[A] YM-2A, [B] YM-11, [C] YM-44, [D] YM-51, [E] YM-6A, [F] MD fraction, [G] YM-52. Changes in body weight and survival in the groups pre-treated with *GF* products are shown.



Discussion

Seven *GF* preparations were administered to the mice for 7 days before exposure to lethal X-irradiation. The pre-treatment with YM-6A resulted in 20% survival on day 30. The remaining six preparations tested here did not have such radioprotective effect. The treatment with YM-52 preparation, composed of the YM-6A and MD fractions (YM-6A:MD=1:1), resulted in survival of one mouse until day 28. No additional effect was observed when the dose of YM-52 was increased (data not shown). These results suggest that the radiomitigative effect of YM-6A was canceled by the presence of MD components which components might have this negative effect.

Previous studies have shown that *GF* β-glucan promotes granulopoiesis by increasing G-CSF production [6-8]. Lin et al. have shown that GF β-glucan activate the production of G-CSF in CB CD33+ monocytes [6]. Matsuda et al. demonstrated that MD-Fraction significantly increases the expression of mRNAs for granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, macrophage colony-stimulating factor (M-CSF), IFN-γ, and IL-12 p40 in splenocytes. It also alleviates the decrease in the number of CFU-GM colonies in cisplatin-treated bone marrow [8]. Moreover, Ito et al. have reported that *GF* β-glucan enhances the granulopoiesis andmobilization of granulocytes and their progenitors by stimulating G-CSF production [7]. As shown in the previous reports, MD preparation of *GF* contains β-1, 6-glucan with β-1, 3-branches [4,8]. Only YM-6A contains both α- and β-glucans. However, we found that MD did not protect the mice exposed to lethal X-irradiation (Fig. 2). Stickney et al have reported that the duration of severe thrombocytopenia appeared to correlates with death to a greater extent than does the duration of severe neutropenia [10]. β-glucans have a potential to promote hematopoiesis; however, they affect mainly granulopoiesis and not thrombopoiesis. Taken together, there is a possibility that some factors other than β-glucans, e.g. β-glucans, promote megakaryopoiesis and thrombopoiesis, enhancing the survival of mice exposed to lethal radiation.

We did not examine the cytokine concentrations, HSC numbers or redox state in each mouse. However, our results indicate that *GF* contains some factors with radiomitigative properties. The preparations used in our study did not affect the growth of non-irradiated control mice (Figure 1), suggesting that the orally administered *GF* preparations are not toxic at the doses used here. Therefore, *GF* can be ingested on a daily basis; it is likely to counter some of the harmful effects of radiation exposure, such as low-dose, long-term exposure.

Acknowledgments

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