Chapter 7

Radiation Protective and Immunopotentiating Effect of Lymphocytes by β-Glucan

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Additional information is available at the end of the chapter

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Abstract

Various intractable diseases have been cured by the development of many new medicines. However, cancer is still a major cause of death. We focused on β-glucan which heat treated Enterococcus faecalis in human intestine, and investigated antioxidant activity, antitumor activity, immune response, etc. In this study, we studied the immunostimulatory effect of β-glucan and its application as a radioprotective agent. As a result, β-D-glucan is known to possess antioxidant activity. Its action is thought to be due to radical scavenger reaction. In our study, β-glucan increased the amount of killer cells and suppressor T cells. Therefore, administration of β-glucan is thought to have an anticancer action. In the future, we hypothesize that β-glucan could be used as an immunopotentiating agent which is effective for the prevention of cancer. From now on, further research will be necessary on the search for cancer cell receptors and to conduct fundamental research on the synergistic effects of radiation therapy. β-glucan showed strong antitumor activity against two kinds of solid carcinoma. Taken altogether, this strongly suggests that β-glucan enhances innate functions of macrophages and NK cells, and as a result, secondarily enhances the immune reaction and suppresses tumor growth.

Keywords: radioprotection, β-glucan, antitumor activity, immunoresponse, IFN-γ, NK cells, TNF-α, lymphocytes

1. Introduction

Previously, various intractable diseases have been cured by the development of many new or alternative medicines approaches. Although there is considerable progress in the development of anticancer drugs, cancer is still the leading cause of death in developed countries. Carcinogenesis induced by various environmental factors has different mechanisms.

Depending on the type of cancer, the therapeutic effect varies and individual differences are also variably large. Therefore, early detection, early treatment and early treatment are urgent [1].

The primary cause of death in Japan is malignant tumors. The incidence of malignant tumors is increasing year by year and one out of three patients die from malignant tumors [2]. Japanese longevity is considered a lifestyle such as ingestion of a lot of fish, ingestion of enzyme foodstuffs and diligence [3, 4]. In any case, malignant tumors have to be destroyed and, at present, there are three main forms of treatment that directly kill the lesion or malignant cells [5].

These three major forms of therapy have severe mental and physical burdens as well as the side effects and weakening of the immune system are also serious problems. In addition, there is no possibility of cessation of treatment, and the motivation for life is also considerably reduced [6–9].

Therefore, research on treatments that exclude the burden on the body as much as possible, such as surgery using a robot and immunotherapy, is underway in recent cancer treatment methods [10, 11].

At the same time, research has also been made to improve disease from the root cause from the viewpoint of oriental medicine. However, it seems that the three major therapies will continue to establish immovable status in the treatment of malignant tumors, and side effects caused by them as well as the reduction of immunity are main problems [3, 12–14].

Radiotherapy is especially important because it is a noninvasive and function-preserving method. And, if the side effects on normal tissues related to treatment can be overcome, it would be one of the most important methods in the future. When hematopoietic tissue is affected by radiation, bacterial and viral infections due to immune system weakness, genetic changes due to cell necrosis and DNA damage, mutations, and cancer occur as side effects [15–17].

For that reason, various radioprotective agents have also been studied. In fact, a radiation protection agent called WR-2721 was developed at the Water Reed Army Hospital in the United States and clinical trials were conducted, but it is no longer commonly used due to side effects such as vomiting and rash [18–20].

Therefore, it is urgent to develop a radiation protection agent with fewer side effects, and approaches focusing on natural substances, which are said to have relatively few side effects.

In recent years, approaches for reducing the side effects of anticancer drugs with natural substances in Japan and Korea are prosperous, among which propolis is drawing attention as a natural anticancer agent and an immunopotentiating agent [19, 21].

Various medicinal ingredients and physiological effects have been reported on this substance. As components thereof, carbohydrates, amino acids, minerals, terpenoids, amino acids and the like are contained and components contained therein are changed by the extraction method.

In the case of general alcohol extraction, numerous lipophilic components such as flavonoids, which are said to be effective ingredients, are extracted, but in the case of the water extraction method devised by Suzuki et al. the levels of flavonoids decrease. However, there are reports that water extraction has higher antitumor effect than alcohol extraction. Antitumor effects are reported for β-(1-3) D glucan, β-(1-6) D glucan [22, 23].

Based on the above information, in this study, we examined the protective effect of β-glucan on lymphocytes exposed to radiation as well as its ability in reducing side effects caused by radiation therapy [24–27]. In case of radiation therapy, it is applied when the radiation sensitivity is high under the condition that the cancer condition is local.

We also examined the effect on tumor growth when combined with radiation therapy. Therefore, this study aims to propose β-glucan as the first step as a new radioprotective agent that can be used at the time of radiotherapy and to contribute data for future development.

2. Materials and methods

2.1. Animals

Mice were purchased from a Japanese SLC company and adjusted to the experimental conditions of this study for 1 week prior to the start of the experiment. The mouse used in this study is a male C3H/Hej mouse. In order to synchronize the purchased mouse, the light was adjusted (12 L:12 D) and kept. Food and water were allowed *ad libitum*.

2.2. Test material

Enteroccous Faecalis 2001®, a glucan product, composed of yeast extract, dextrin and gelatin was supplied by Nihon BRM Co. Ltd. (Tokyo, Japan) and β-1,3 glucan as an active ingredient was contained at the ratio of 6.5 mg/g of the product. *Enteroccous Faecalis 2001*® is hereafter described as β-glucan throughout this paper. Glucan was suspended in physiological saline at concentrations of 2, 4 and 8% (w/v).

2.3. Radio-protective effect

Each mouse was intraperitoneally injected with β-glucan at a dose of 200, 400, 2800 mg/kg/day at 1 day intervals for 2 weeks. The sham control group of mice received an equal volume of normal saline. After the final injection, the mice were irradiated with 2 Gy of X-ray. Whole body irradiation was performed with a dose of 8 Gy (dose rate 1.12 Gy/min) using an X-ray irradiation device (MG226/4.5, Phillips, Inc., Tokyo). Body weight and number of surviving animals were monitored daily.

2.4. Antitumor effect

SSC-7 carcinoma cells were inoculated subcutaneously (5×10^5) into the right femur of mice.

After the average long diameter of the tumors reached 5 mm, mice were intraperitoneally injected with β-glucan suspended in physiological saline at a dose of 200, 400 and 800 mg/kg/day for 5 consecutive days. Control mice received an equal volume of normal saline. At the given time point, the major and minor axes of the tumor were measured with a caliper and the tumor volume was calculated by the following equation; $V = (\pi/6)\ ab^2$, a: major axis length, b: minor axis length.

2.5. Leukocyte and lymphocyte counts

The β-glucan suspended in physiological saline was intraperitoneally injected into mice at doses of 200, 400, 800 mg/kg. Control mice received an equal volume of normal saline. After the injection, in order to measure the number of white blood cells and lymphocytes using an automated hemocytometer (Celltac-α, MEK-6318, Nihon kouden Co. Ltd., Tokyo), a blood sample was transferred from the tail vein into a heparinized tube.

2.6. NK cell activity

The β-glucan suspended in physiological saline was intraperitoneally injected at a dose of 400 and 800 mg/kg for 2 weeks at intervals of 1 day. Vehicle control mice received an equal volume of normal saline. Spleen cells were prepared to measure NK cell mediated cytotoxicity by ^{51}C-release from labeled AC-1 cells (2×10^4 cells) after the final injection. Briefly, 51Cr labeled YAC-1 cells (2×10^4 cells) were added to various dilutions of splenocyte suspensions in flat bottom microplates. The mixture was incubated for 4 h at 37°C in a CO_2-incubator. The radioactivity released to the supernatant was counted with a γ-counter and the magnitude of cell lysis calculated based on the average radioactivity of the control group was defined as NK cell activity.

2.7. Statistical analysis

Significance of the difference in each parameter among groups was assessed by Tukey's multiple comparison tests following analysis of variance. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Radio-protective effect and leukocyte and lymphocyte counts

The survival of irradiated mice is summarized in **Figures 1–5**. All of the animals in the irradiated control group died from the 5th day to the 7th day following irradiation. Glucan injected intraperitoneally prolonged the survival of mice 80% on the 7th day at the doses of 200 mg/kg.

The number of leukocytes increased with time at least up to 24 h after each repeated dose of glucan in a dose-dependent manner. The lymphocyte counts also showed a similar tendency to increase as in the leukocyte counts.

3.2. Antitumor effect

Figure 6 shows anticancer effect of various β-glucan concentrations. The tumor of the control group grew with time, whereas glucan injected intraperitoneally made tumor growth delay significantly in a dose-dependent manner **Figure 3**.

3.3. NK activity

Figure 7 shows NK activity of various β-glucan concentrations. NK cell activities in mice are shown in **Figure 7**. The NK activity increased significantly about twofold to threefold after each repeated dose of glucan (400 and 800 mg/kg).

3.4. Discussion

In the present study, we have found that β-glucan showed antioxidant activity. Previously, Ekaterini et al. have reported that β-glucan shows antioxidant activity [28], suggesting that β-glucan may be responsible for the activity that we found in our study. β-Glucan is well known for its radioprotective and antitumor effect *in vivo* [29–32], and these effects were reproduced in this study.

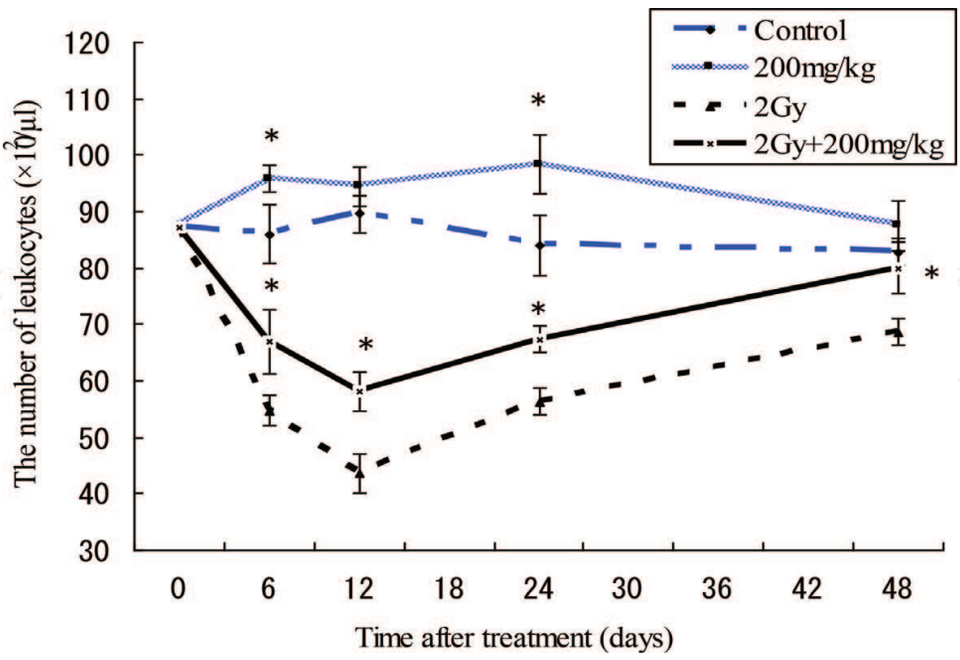

Figure 1. Leukocyte counts on different days after irradiation in mice of different groups. The number of leukocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. *Statistically significant ($p < 0.05$) from the control group.

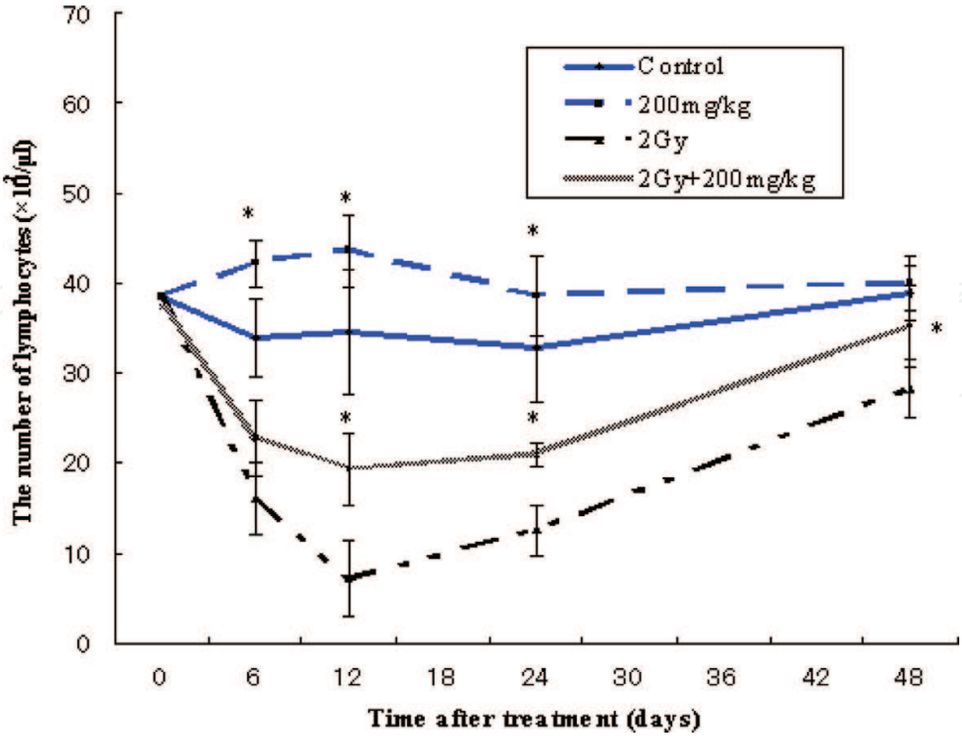

Figure 2. Lymphocyte counts on different days after irradiation in mice of different groups. The number of lymphocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. *Statistically significant ($p < 0.05$) from the control group.

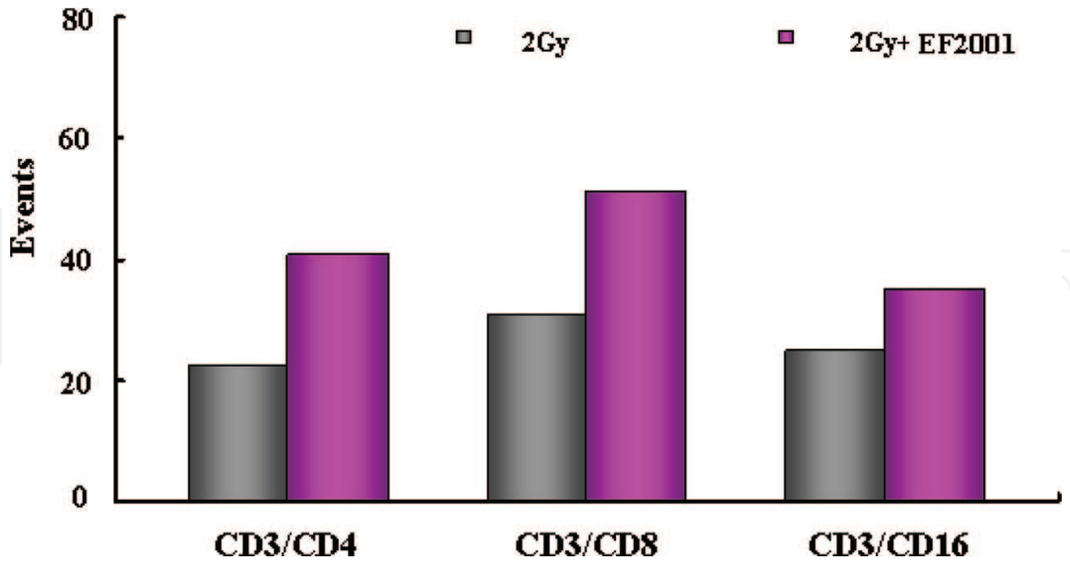

Figure 3. The increased percentage of CD4+, CD8+ and CD16+ T-lymphocytes in PBLs compared to the experimental data baselines of the groups. The unit is in percentage (%). Significantly different from *$p < 0.05$ control group vs. β-glucan groups by Dunnett's test.

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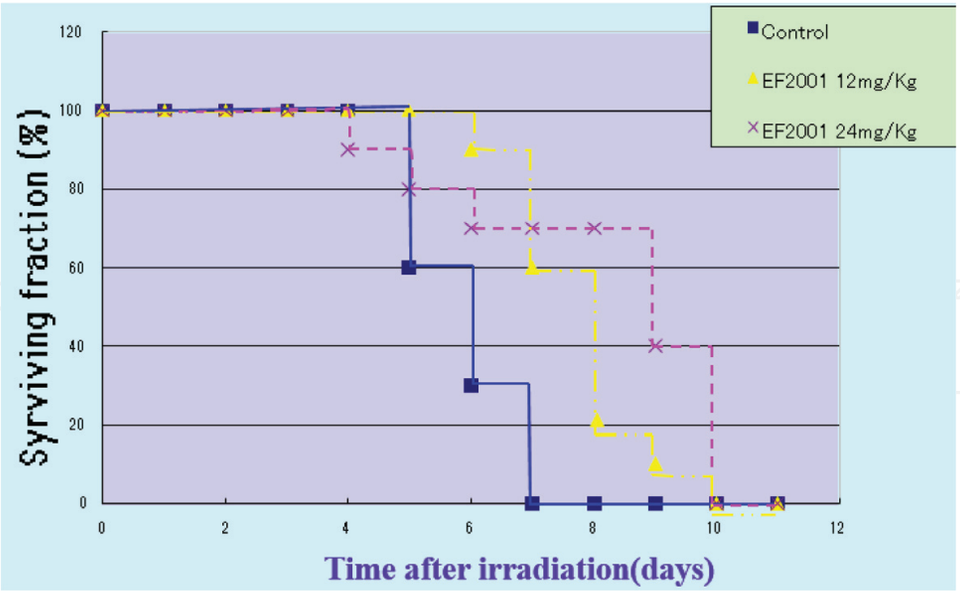

Figure 4. Survival after irradiation. Surviving fraction was increased after injection of β-glucan.

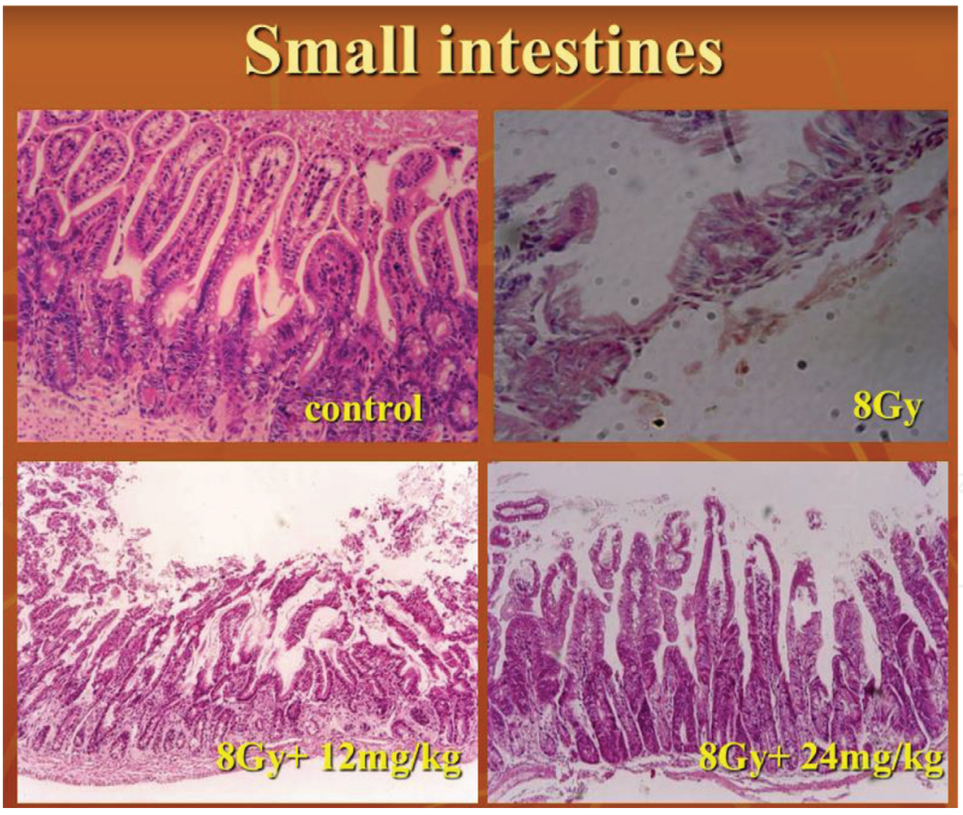

Figure 5. Radiation protection effect of mucosal damage of small intestine to β-glucan of both doses of 12 and 24 mg/kg concentration.

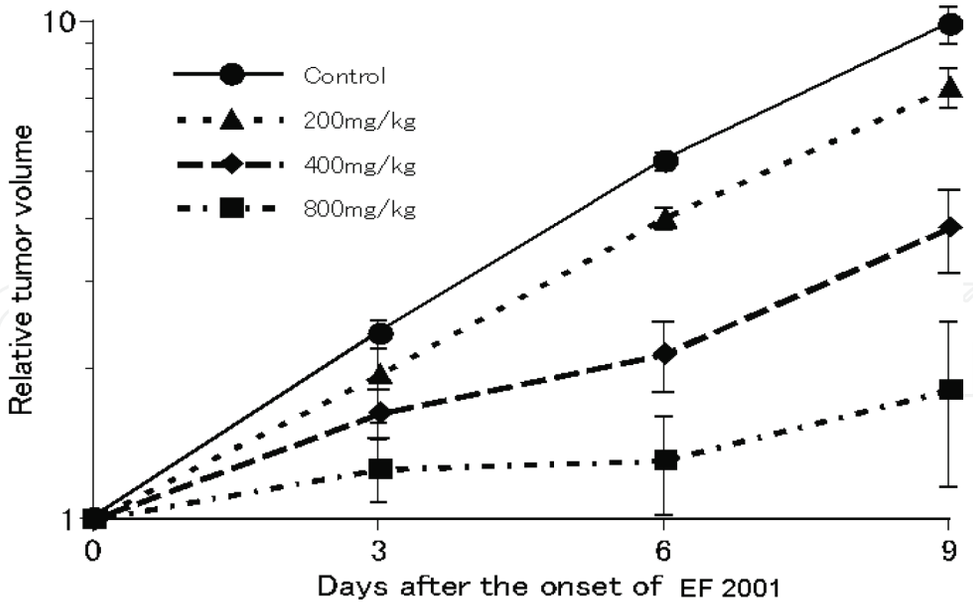

Figure 6. Effect of β-glucan on the tumor growth in mice inoculated with SSC-7 carcinoma cells. Groups of 10 mice were subjected to different treatment. Results represent means ± S.D. *Statistically significant ($p < 0.05$) from the control group.

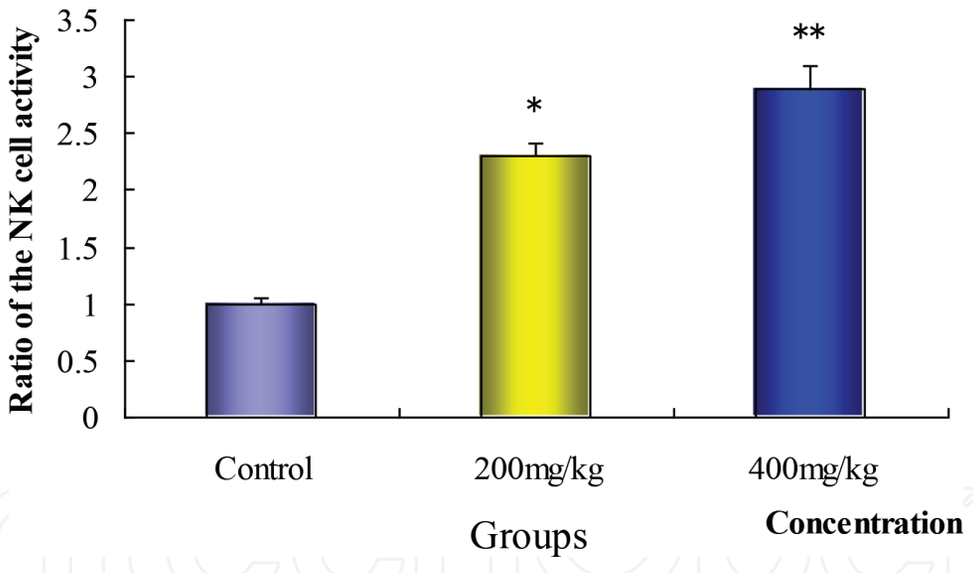

Figure 7. Repeated dose effect of β-glucan on the NK activity in mice. Groups of 10 mice each were subjected to each treatment. Results represent means ± S.D. *Statistically significant ($p < 0.05$) from the control group. **Statistically significant ($p < 0.01$) from the control group.

To confirm the elucidation mechanism by which β-glucan exerts these effects, the number of white blood cells and lymphocytes was monitored as a hematopoietic effect. Furthermore, NK cell activity was measured as an immunological parameter. The results of these parameters demonstrated that the radioprotective effect of β-glucan is mediated, at least in part, by hematopoietic effects in irradiated mice. This is because the leukocyte and lymphocyte counts

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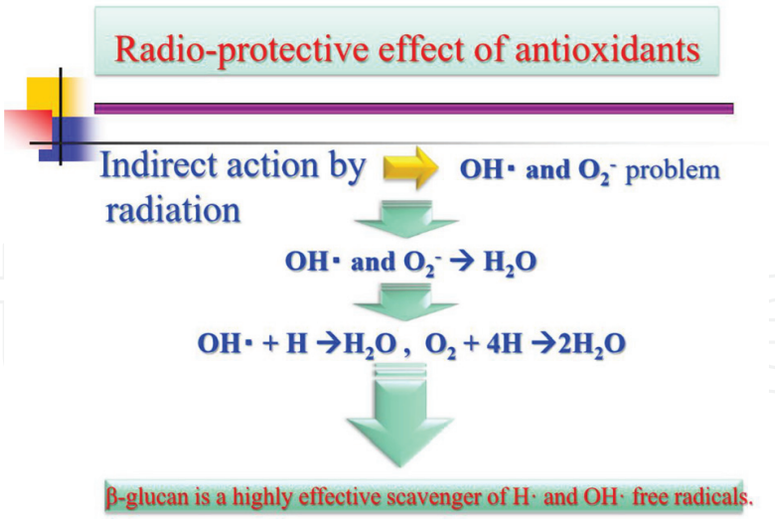

Figure 8. This slide shows a mechanism of radiation protection effect of various β-glucan concentrations.

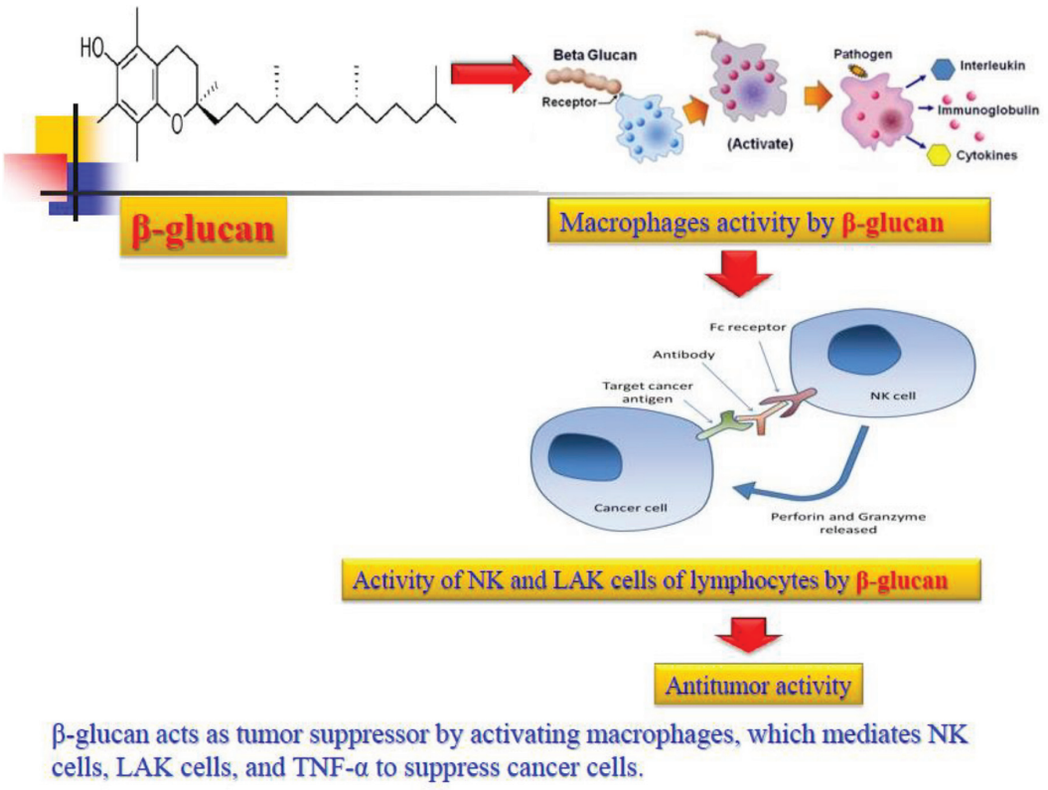

Figure 9. This slide demonstrates a mechanism of activity of NK and LAK cells of lymphocytes by various β-glucan concentrations.

increased with a single administration of β-glucan. In addition, the enhanced immunological activity as seen by the increase in NK cell activity by β-glucan appears to play a role in the prevention of secondary infections associated with radiation.

Natural killer (NK) and lymphokine-activated killer (LAK) cells are well known to be associated with cytotoxic effects on various kinds of tumor cells [33, 34]. The reason why the immature immune response of young mice was activated to the same level as observed in adult mice is that the various components in the sample used in this experiment most probably activated IL-2 and IL-6 of the immune system. IFN-γ, cytokines and the like are multifunctional factors showing antiviral effect, inhibition of cell proliferation, antitumor effect, macrophage activation, enhancement of NK cell activation, regulation of immune response, and differentiation induction.

Specifically, high levels of IFN-γ were measured in mice bearing the S-180 carcinoma, after administration of β-glucan [35–38].

IL-4 is mainly associated with a class switch of IgM to IgG. IFN-γ and IL-4 are related to differentiation of Th1 and TH2, respectively, and these cytokines suppress each other [37–39].

When antitumor action was examined using two kinds of sarcoma (Ehrlich solid carcinoma and Sarcoma-180 carcinoma), tumor-suppressive ratios after treatment with β-glucan was 83%. When Sarcoma-180 solid carcinoma was used, tumor-suppressive ratios was 60%. Thus, β-glucan showed strong antitumor activity against two kinds of solid carcinoma [40, 41].

Therefore, there is the possibility that after treatment with β-glucan, in which no antioxidant activity is found, the balance between NF-κB and I-κB is lost, and the apparent tumor-suppressive ratio is low due to suppression of apoptosis of tumor cells. Thus, it is possible that β-glucan restores the balance between NF-κB and I-κB, and induces apoptosis and tumor suppression secondarily [42].

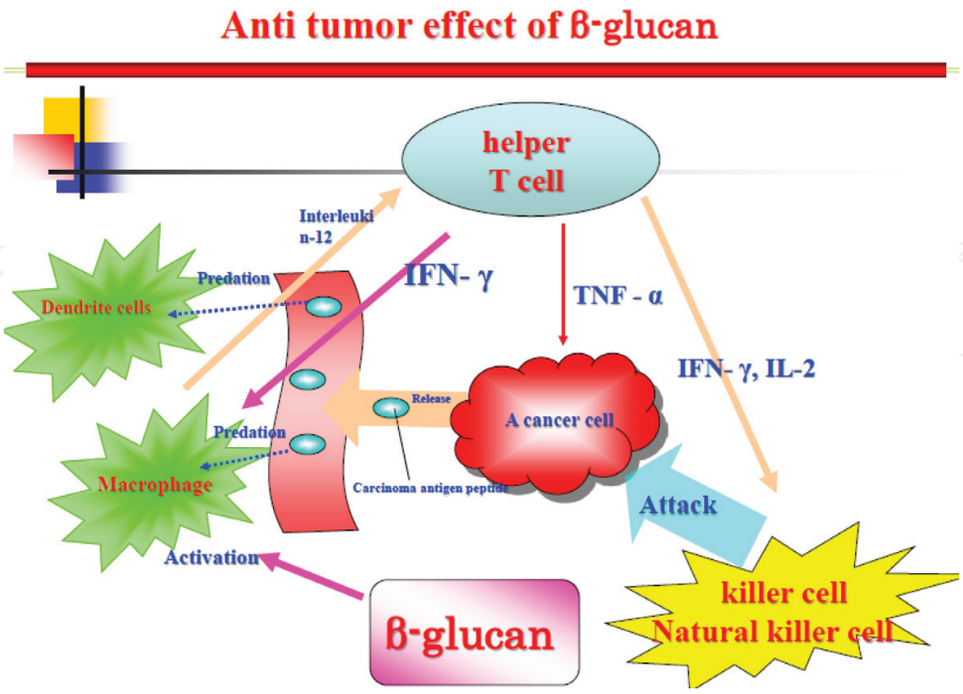

Figure 10. This slide shows a mechanism of activity of antitumor effect by β-glucan.

Therefore, increased activity of NK by β-glucan contributes most probably to attenuated tumor growth in tumor-bearing mice. Based on these data, glucan is expected to be a promising agent for the treatment of cancer patients receiving radiotherapy (**Figures 8–10**).

4. Conclusion

In this study we have evaluated the anticancer properties of β-glucan using a mouse model. β-glucan was found to upregulate CD8+ lymphocytes and leukocytes as well as reducing tumor volume and mass. We believe two phenomena may be correlated but further research is required to elucidate the exact role of β-glucan. Nonetheless, it may be possible to suggest that β-glucan could be used as a potential anticancer agent. β-Glucan was also found to improve recovery rate of lymphocytes and leukocytes following irradiation. We believe this may be due to antioxidant properties of β-glucan suggesting its possible use as an alternative radioprotective agent with reduced side effects.

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