原　著

Radioprotection by yeast-derived β-glucan in mice

Yeunhwa GU[1)], Hiroyo KONDO[2),3)], Takashi NAKAMURA[1)], In-Suk CHOI[1)], Takeo HASEGAWA[1)], Masami OSHIMA[1)], Takenori YAMASHITA[1)], Ikukatsu SUZUKI[2)], Toshihiro MAENAKA[1)], Ihil-Bong CHOI[4)], Takehiko MORI[1)] and Torao ISHIDA[3)]

[1)]Department of Radiological Technology, Suzuka University of Medical Science

[2)]Department of Clinical Nutrition, Faculty of Health Science, Suzuka University of Medical Science

[3)]Institute of Traditional Cinese Medicine

[4)]Department of Radiation Oncology, Catholic University Medical College, Seoul, Korea

Key Words: Key Words : β-glucan, redioprotective effect, anti-tumor effect

Abstract

Intraperitoneal injection of β-glucan greatly reduces mortality of mice exposed to whole body X-ray radiation and tumor growth in tumor bearing mice. Since the leukocyte and lymphocyte number was increased by a single dose of β-glucan, the radioprotective effect of β-glucan is probably mediated at least in part by a hemopoietic action in irradiated mice. In addition, both of the Natural killer (NK) and lymphokine activated killer (LAK) activity increased significantly by repeated dose of β-glucan. Augmented immunological activity as seen in increased NK and LAK activity by β-glucan seems to play a role in preventing secondary infections associated with irradiation, and to contribute probably to attenuated tumor growth in tumor-bearing mice through enhanced anti-tumor immunity. From these, β-glucan is expected to be promising for the treatment of cancer patients receiving radiotherapy.

1. Introduction

Depression of hemopoiesis and immunity following radiotherapy to treat cancer is a well-known phenomenon[1),2)]. Therefore, the strategies to protect cancer patients from the disadvantage of radiation without compromising anticancer effects are of considerable concerns in radiotherapy[3),4)], and researches have been conducted to develop effective, inexpensive, and non-toxic agents for radioprotection[5)].

β-Glucan, frequently termed as a biological response modifier (BRM), is a well-documented agent exerting radioprotective and anti-tumor effects[6),7)] through macrophage activation, augmented reticuloendothelial system activity, stimulated immunity, and enhanced hemopoiesis[8)-13)]. Furthermore, more detailed mechanisms by which β-glucan exerts immune-stimulating effects have recently been clarified. Namely, it functions through binding to CR3 (CD11b/CD18) of neutrophils, macrophages, and NK cells for cytotoxicity of iC3b-opsonized tumor cells[14)-17)]. The present study was focused on investigating the use of β-glucan as a radioprotective and anti-tumor agent in experimental animal models in relation to its hemopoietic and immune-stimulating effects.

2. Materials and methods

2.1. Animals

Male C3H/Hej mice purchased from Japan SLC (Shizuoka, Japan) were used at 7 weeks of age. Mice were housed with controlled lightning (12L : 12D) and food and water were given *ad libitum*. All mice were acclimated to laboratory conditions for 1 week before experimentation.

2.2. Test material

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

2.3. Radio-protective effect

Mice were intraperitoneally injected with β-glucan suspended in physiological saline at a dose of 200, 400 or 800mg/kg/day for two weeks at one-day intervals. The vehicle-control mice received an equivalent volume of physiological saline. After the final injection, mice were exposed to X-ray radiation. Whole body radiation exposure was carried out at a dose of 8 Gy (a dose rate of 1.12 Gy/min) using a X-ray irradiation device (MG226/4.5, Phillips, Inc. Tokyo). Body weight and the number of surviving animals were daily monitored.

2.4. Anti-tumor effect

Mice were subcutaneaously inoculated with 5×10^5 SSC-7 carcinoma cells at the right femur. After the tumor size attained 5 mm in the average major axis, mice were intraperitoneally injected with β-glucan suspended in physiological saline at a dose of 200, 400 or 800mg/kg/day for five consecutive days. The vehicle-control mice received an equivalent volume of physiological saline. At the given time points, a major and a minor axis of the tumor were measured by a caliper and 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

Mice were intraperitoneally injected with β-glucan suspended in physiological saline at a dose of 200, 400 or 800mg/kg. The vehicle-control mice received an equivalent volume of physiological saline. After the injection, blood samples were obtained from caudal vein into heparinized tubes at given time points for measuring leukocyte and lymphocyte counts using an automated hematology analyzer (Celltac-α MEK-6318, Nihonkouden Co., Ltd. Tokyo).

2.6. NK activity

Mice were intraperitoneally injected with β-glucan suspended in physiological saline at a dose of 400 or 800mg/kg for two weeks at one-day intervals. The vehicle-control mice received an equivalent volume of physiological saline. Twenty-four hours after the final injection, spleen cells were prepared for measuring NK cell-mediated cytotoxicity by ^{51}C-release from labeled YAC-1 cells. Briefly, ^{51}Cr-labelled YAC-1 cells (2×10^4 cells) were added to various dilutions of spleen cell suspension in flat-bottomed microplates. The mixtures were incubated at 37℃ for 4 hr in a CO_2-incubator. The radioactivity released into the supernatant was counted by a γ-counter, and the magnitude of cytolysis calculated based on the average radioactivity of the control group was defined as NK activity.

2.7. LAK activity

Mice were intraperitoneally injected with β-glucan suspended in physiological saline at a dose of 400 or 800mg/kg for two weeks at one-day intervals. The vehicle-control mice received an equivalent volume of physiological saline. Twenty-four hours after the final injection, spleen cells were prepared for measuring LAK cell-mediated cytotoxicity by ^{51}Cr-elease from labeled EL-4 cells. Briefly, ^{51}Cr-labelled EL-4 cells (2×10^4 cells) were added to various dilutions of spleen cell suspension in flat-bottomed microplates. The mixtures were incubated at 37℃ for 4 hr in a CO_2-incubator. The radioactivity released into the supernatant was counted by a γ-counter, and the magnitude of cytolysis calculated based on the average radioactivity of the control group was defined as LAK activity.

2.8. Statistical analysis

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

3. Results

3.1. Radio-protective effect

The survival of irradiated mice is summarized in Table 1 and Fig. 1. All of the animals in the irradiated control group died from the 5th day to the 7th day following irradiation. β-Glucan injected intraperitoneally prolonged the survival of mice; as shown in the Fig. 1 80% of mice still survived on the 7th day at the both doses of 400 and 800mg/kg.

Body weight loss was remarkable, and X-ray alone group showed decrease of higher than 30% after irradiation in the sixth day whereas the weight gain that the control group. Which we did not irradiate was favorable was seen. However, it was about 20% decrease, and inhibition of weight loss was seen in the β-Glucan treated group in comparison with control group in post radiation the sixth day. Weight loss was about 20% with 800mg/kg treated group in post radiation the ninth day, and inhibitory effect of weight loss was seen most in that. In addition, as for the β-Glucan 800mg/kg treated group, a meaningful

Table 1. Effect of β-glucan on the survival rate of mice exposed to whole body radiation. Groups of ten mice each were subjected to each treatment.

The course days(days)	X-ray alone(%)	400mg/kg(%)	800mg/kg(%)
0	100	100	100
1	100	100	100
2	100	100	100
3	100	100	100
4	100	100	100
5	60	100	100
6	30	100	100
7	0	80	80
8	−	60	80
9	−	30	40
10	−	0	10
11	−	−	0

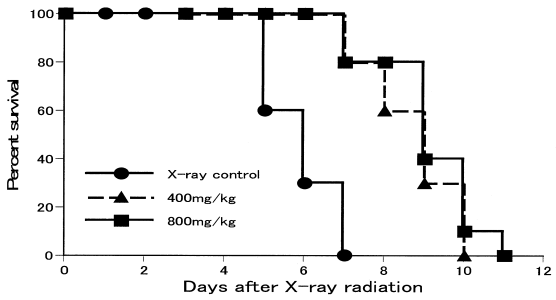

Fig. 1. Effect of β-glucan on the mortality of mice exposed to whole body radiation. Groups of ten mice each were subjected to each treatment.

difference ($P < 0.005$) was accepted in comparison with X-ray alone group in post radiation two or three, four or five, the sixth day.

3.2. Anti-tumor effect

The tumor growth rates in mice inoculated with SSC-7 carcinoma cells are summarized in Table 2 and Fig. 2. The tumor of the control group grew with time, whereas β-glucan injected intraperitoneally made tumor growth delayed significantly in a dose-dependent manner.

As level of β-Glucan became 400mg/kg from 200mg/kg, 800kg/kg from 400mg/kg-delayed growth of a tumor, and level 800mg/kg treated group was

Table 2. The days that is necessary if we let you double size of 3 tumors and the ratio.

Level	Days	The ratio
Control	2.4	1.0
200mg/kg	3.1	1.3
400mg/kg	4.0	1.7
800mg/kg	5.2	2.2

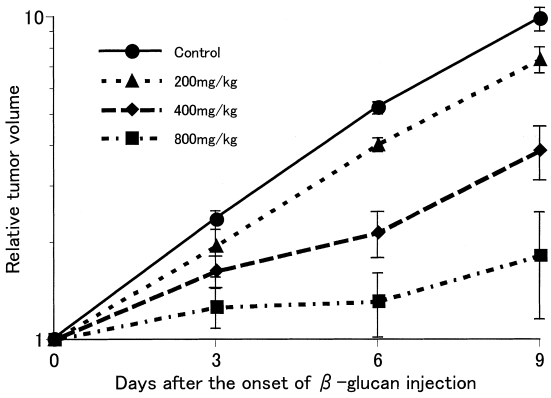

Fig. 2. Effect of β-glucan on the tumor growth in mice inoculated with SSC-7 carcinoma cells. Groups of ten mice each were subjected to each treatment. Results represent means ± S. D. *Statistically significant ($P < 0.05$) from the control group.

able to delay growth of a tumor most. In addition, control group and a meaningful difference ($P < 0.005$) produced the 800mg/kg treated group in all measurement time. As for the days that were necessary if we let you double size of a tumor, in β-Glucan 200mg/kg group 3.1, as for day, 400mg/kg, and 800mg/kg group 5.2 were days on 4th. When they assumed the growth days of control group 1.0 and compared it with each treated group, as for 200mg/kg, as for 1.3 times, 400mg/kg, 1.7 times, 800mg/kg showed a value of 2.2 times each.

3.3. Leukocyte and lymphocyte counts

The number of blood leukocytes and lymphocytes in normal mice are summarized in Table 3, 4, Fig. 3 and 4, respectively. The number of leukocytes increased with time at least up to 24 hr after each repeated dose of β-glucan in a dose-dependent manner. The lymphocyte counts also showed a similar tendency as in the leukocyte counts.

At 6, 12, 24 hours and leukocytes count increased after administration, and β-Glucan level 400, the 800mg/kg treated group came back to an original value 48 hours later. We compared it with control group in 800mg/kg treated group in 400mg/kg treated group administration 6, 12, 24 hours later administration 12, 24 hours later, and a meaningful difference ($P < 0.005$) occurred. This, as for the increase of leukocytes, 800mg/kg treated group showed the superior value and we compared it with control group and we were greatest and showed a value (24 hours later) of 1.2 times. On the other hand, an increase tendency was seen in the 200mg/kg treated group after administration six hours later, but appearing decreased 12 hours later. We increased in the back for 24 hours, but the significant difference with control group was not recognized afterwards in all time. Same as leukocytes, at 6, 12, 24 hours and leukocytes increased after administration, β-Glucan level 400, the 800mg/kg treated group came back to an original

Table 3. The mean and standard deviation of a change value with time of leukocytes

An elapsed time	Control		200mg/kg		400mg/kg		800mg/kg	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
0	87.1	−	87.1	−	87.1	−	87.1	−
6	86.0	5.23	95.8	2.32	97.2	2.71	106.0	5.78
12	89.6	3.32	97.2	3.45	99.2	3.47	111.9	3.46
24	84.0	5.22	98.3	5.23	107.9	2.36	115.0	2.42
48	82.8	2.38	87.5	4.49	86.3	2.33	84.9	4.52

Unit ; ($*10^2/\mu l$)

Table 4. The mean and standard deviation of a change value with time of lymphocytes

An elapsed time	Control		200mg/kg		400mg/kg		800mg/kg	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
0	38.5	−	38.5	−	38.5	−	38.5	−
6	38.4	4.28	42.0	2.55	54.7	4.04	57.9	4.18
12	32.0	7.00	42.8	4.11	53.5	4.25	64.2	4.06
24	33.8	5.94	43.8	4.45	59.3	2.85	64.7	1.32
48	36.7	2.96	38.2	2.97	40.0	3.23	35.3	4.57

Unit ; ($*10^2/\mu l$)

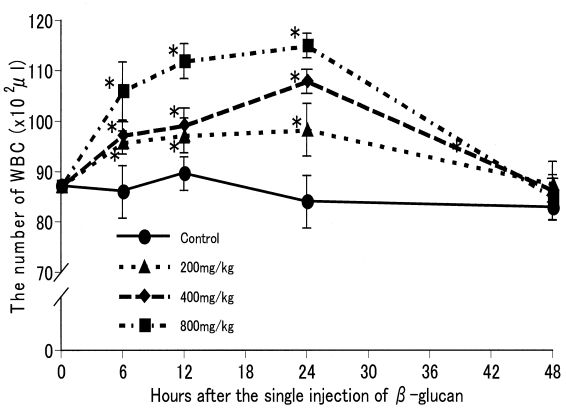

Fig. 3. Single dose effect of β-glucan on the blood WBC counts in mice. Groups of ten mice each were subjected to each treatment. Results represent means ± S. D. * Statistically significant ($P < 0.05$) from the control group.

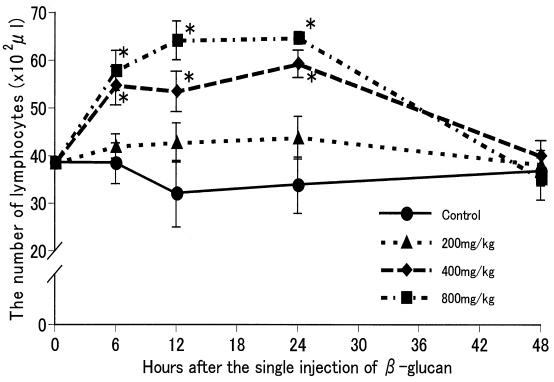

Fig. 4. Single dose effect of β-glucan on the blood lymphocyte counts in mice. Groups of ten mice each were subjected to each treatment. Results represent means ± S. D. * Statistically significant ($P < 0.05$) from the control group.

value 48 hours later. We compared it with control group in 400, 800mg/kg treated group administration 6, 12, 24 hours later, and a meaningful difference ($P < 0.005$) occurred. This, as for the increase of lymphocytes, 800mg/kg treated group showed the superior value and we compared it with control group and we were greatest and showed a value (24 hours later) of 1.7 times. On the other hand, 200mg/kg treated group did not show lymphocytes, and significant difference with control group did not occur, too.

3.4. NK activity and LAK activity

NK activity and LAK activity in mice are shown in Figs. 5 and 6, respectively. Both of the NK and LAK activity increased significantly about twofold to threefold after each repeated dose of β-glucan (400 and 800 mg/kg).

When we standardized control group with 1 and compared it with 400, 800mg/kg treated group, 2.5 times, natural killer cell activity of 1.7 times were observed each. In addition, a meaningful difference ($P < 0.01$) was obtained to a treated group in comparison with control group.

When we standardized control group with 1 and compared it with 400, 800mg/kg treated group, 2.8 times, lymphokine-activated killer cell activity of 2.0 times were observed each. In addition, a meaningful difference ($P < 0.01$) was obtained to a treated group in comparison with control group.

4. Discussion

β-Glucan is well known to exert radioprotective effect and anti-tumor effect *in vivo*[6),7)], and these effects were reproduced in this study. To confirm the elucidative mechanisms by which β-glucan exerts these effects, the number of leukocyte and lymphocyte was monitored as a hemopoietic action. Furthermore, NK and LAK activity were measured as immunological parameters. The results of these parameters demonstrated that the radioprotective effect of β-glucan is probably mediated at least in part by a hemopoietic action in irradiated mice since the leukocyte and lymphocyte number was increased by a single dose of β-glucan. In addition, augmented immunological activity as seen in increased NK and LAK activity by β-glucan seems to play a role in preventing secondary infections associated with irra-

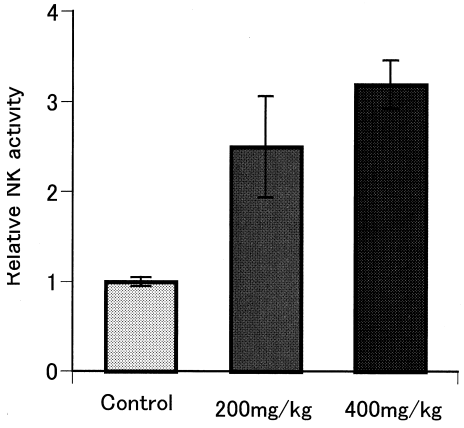

Fig. 5. Repeated dose effect of β-glucan on the NK activity in mice. Groups of ten mice each were subjected to each treatment. Results represent means ± S. D. * Statistically significant ($P < 0.05$) from the control group.

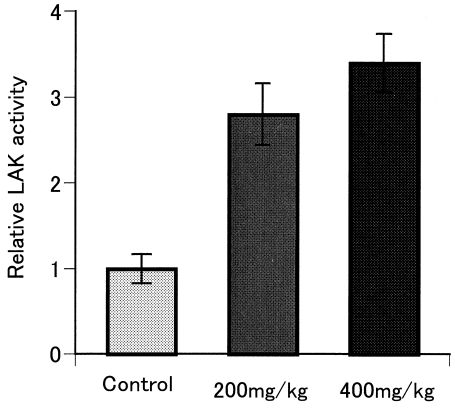

Fig. 6. Repeated dose effect of β-glucan on the LAK activity in mice. Groups of ten mice each were subjected to each treatment. Results represent means ± S. D. * Statistically significant ($P < 0.05$) from the control group.

Table 5. Natural killer cell activity value

	Control	400mg/kg	800mg/kg
Activity	1	2.5	3.19
Standard deviation	0.05	0.56	0.27

Table 6. Lymphokine-activated killer cell activity value

	Control	400mg/kg	800mg/kg
Activity	1	2.8	3.4
Standard deviation	0.17	0.36	0.34

diation. Natural killer (NK) and lymphokine activated killer (LAK) cells are well known to be associated with cytotoxic effect on various kinds of tumor cells[18)-22)]. Therefore, increased activity of NK and LAK by β-glucan contributes probably to attenuated tumor growth in tumor-bearing mice. From these, β-glucan is expected to be promising for the treatment of cancer patients receiving radiotherapy.

5. References

1) Blackett, N. M., Marsh, J. C., Gordon, M. Y., Okell, S. F. and Aguado, M.: Simultaneous assay by six methods of the effect on hemopietic precursor cells of adriamycin, methyl-CCNU, ^{60}Co rays, vinblastine, and cytosine arabinoside. Exp. Hematol. 6: 2-12, 1978

2) Lohrmann, H. P. and Schreml, W.: Cytotoxic drugs and the granulopoietic system. Springer Verlag, Berlin, 1982

3) Umadevi, P.: Normal tissue protection in cancer therapy: progress and prospects. Acta. Oncol. 37: 247-251, 1998

4) Nair, C. K. K., Parida, D. K. and Nomura, T.: Radioprotectors in radiotherapy. J. Radiat. Res. 42: 21-37, 2001

5) Weiss, J. F.: Pharmacological approaches to protection against radiation induced lethality and other damages. Environ. Health Perspect. 105: 1473-1478, 1997

6) Kasai, S., Fujimoto, S., Nitta, K., Baba, H. and Kunimoto, T.: Antitumor activity of polymorphonuclear leukocytes activated by a β-1, 3-D-glucan.

J. Pharmacobio-Dyn. 14 : 519–525, 1991

7) Patchen, M. L. and MacVittie, T. J. : Comparative effects of soluble and particular glucans on survival in irradiated mice. J. Biol. Res. Modifiers 5 : 45–60, 1986

8) Wools, W. R. and DiLuzio, N. R. : The phagocytic and proliferative response of the RES following glucan administration. J. Reticuloendothel. Soc. 1 : 160–169, 1964

9) Diluzio, N. R., Pisano, J. C. and Saba, T. M. : Evaluation of the mechanism of glucan-induced stimulation of the reticuloendothelial system. R. Reticuloendothel. Soc. 7 : 731–742, 1970

10) Wooles, W. R. and DiLuzio, N. R. : Influence of RE hyperfunction on bone marrow transplantation. Am. J. Physiol. 203 : 404–408, 1962

11) Burgaleta, C. and Golde, D. W. : Effect of glucan on granulopoiesis and macrophage genesis in mice. Cancer Res. 37 : 1739–1742, 1977

12) Patchen, M. L. and Lotzova, E. : Modulation of murine hemopoiesis by glucan. Exp. Hematol. 8 : 409–422, 1980

13) Patchen, M. L. and MacVittie, T. J. : Dose-dependent responses of murine pluripotent stem cells and myeloid and erythroid progenitor cells following administration of the immunomodulating agent glucan. Immunopharmacology 5 : 303–313, 1983

14) Vetvicka, V., Thornton, B. P. and Ross, G. D. : Soluble beta-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J. Clin. Invest. 98 : 50–61, 1996

15) Xia, Y., Vetvicka, V., Yan, J., Hanikyrova, M., Mayadas, T. and Ross, G. D. : The β-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. J. Immunol. 162 : 2281–2290, 1999

16) Yan, J., Vetvicka, V., Xia, Y., Coxon, A., Carroll, M. C., Mayadas, T. N. and Ross, G. D. : β-Glucan, a "specific" biologic response modifier that uses antibodies to target tumors for cytotoxic recognition by leukocyte complement receptor type 3 (CD11b/CD18). J. Immunol. 163 : 3045–3052, 1999

17) Cheung, N. K., Modak, S., Vickers, A. and Knuckles, B. : Orally administered β-glucans enhance anti-tumor effects of monoclonal antibodies. Cancer Immunol。Immunother. 51 : 557–564, 2003

18) Hoover, R. G. and Kornbluth, J. : Immunoregulation of murine and human myeloma. Hematol. Oncol. Clin. North Am. 6 : 407–424, 1992

19) Rossi, A. R., Pericle, F., Rashleigh, S., Janiec, J. and Djeu, J. Y. : Lysis of neuroblastoma cell lines by human natural killer cells activated by interleukin-2 and interleukin-12. Blood 83 : 1323–1328, 1994

20) Karimine, N., Arinaga, S., Inoue, H., Nanbara, S., Ueo, H. and Akiyoshi, T. : Lymphokine-activated killer cell function of peripheral blood mononuclear cells, spleen cells and regional lymph node cells in gastric cancer patients. Clin. Exp. Immunol. 96 : 484–490, 1994

21) Asano, H., Kobari, M., Yusa, T., Kawakami, K. and Matsuno, S. : Effect of LAK cells and BRM on the growth of pancreatic cancer cells injected into nude mice. Nippon Geka Gakkai Zasshi 95 : 678–688, 1994

22) Brittenden, J., Heys, S. D., Ross, J. and Eremin, O. : Natural killer cells and cancer. Cancer 77 : 1226–1243, 1996