

[Original Article]

Radiation Protection Effect for EF 2001 (*Enterococcus Faecalis* 2001)

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Summary

Radiation protection from death and stimulating leukocyte recovery by oral administrations consecutively of EF 2001 (*Enterococcus Faecalis* 2001), 200 mg/kg and 400 mg/kg b.w., once a day, before whole-body x-rays irradiation was confirmed by tests with C3H mice, meanwhile, its radioprotective actions compared to immunological enhancement. Based on the studies of survival, behavior of hematograms, and numbers of lymphocytes, whole body following irradiation, it was demonstrated that EF 2001 was an effective radioprotector. The survival of irradiated mice protected by EF 2001 was significantly increased and statistically higher than that of mice pre-treated with oral administration. After administration of EF 2001, stimulated recovery of leukocyte and lymphocytes counts were observed in mice pre-treated with EF 2001. All above-mentioned results were similar to those in mice protected by EF 2001, but the protecting actions of EF 2001 on promoting recovery of nucleated cells and leukocyte counts were significantly higher than those of EF 2001. It could be deduced that the uncertainly radioprotective action against death is induced by a possible process of enhanced regeneration of the leukocyte stem cells due to not only strengthened radioresistance and increased numbers of remained leukocyte cells, but also enhanced post-irradiation repair or promoted proliferation of the leukocyte stem cells. This effect of EF 2001 may have some therapeutic implications for radiation-induced injuries. We can analyze a result of this study than this thing as follows. In addition, we think that indicating the activation of cell-mediated immune responses.

Key words: EF2001(*Enterococcus faecalis* 2001), Lymphocyte, Radiation protection

Introduction

The hematopoietic system as well as the hemocytes is known to be sensitive to radiation, and low doses of radiation can induce damage. Radioprotective agents are those are administered before exposure to ionizing radiation to reduce the damaging effects, including radiation induced lethality.¹⁾ Many synthetic or natural agents have been investigated in the recent past years for their efficacy to protect against radiation injuries.²⁾ Among the radioprotective

compounds, estrogens have been extensively studied. Either estradiol, belonging to the natural estrogens, or the synthetic estrogens like diethylstilbestrol exerted radioprotective actions on radiation sickness of experimental animals including increasing the survival and accelerating the recovery of hematopoiesis.³⁾ Moreover, estrogens also ameliorated hematopoietic suppression induced by cancer radiotherapy or chemotherapy in the clinic.⁴⁾ However, the inherent toxicities of these agents at the

radioprotective concentration warranted further search of a safer and effective radioprotector.⁵⁾ EF 2001 (*Enterococcus faecalis* 2001; EF 2001), a naturally occurring β -glucan found in *Enterococcus faecalis*.⁶⁾ Many studies have demonstrated that EF 2001, as one of the most important phytoestrogens, had no toxicity on human health at the pharmacological concentration and possessed potential properties to act as both an estrogen and anti-estrogen, inhibit the activities of tyrosine kinase and DNA topoisomerase II, improve immune system.⁷⁾ Consequently, it has gained increasing attentions because of its association with beneficial effects for persons with breast cancer, prostate cancer, cardiovascular disease, high cholesterol levels and osteoporosis.⁸⁾ Moreover, the isoflavone was an effective antioxidant, which could eliminate the free radicals and boost the antioxidant enzymes activities. So that it may provide protection against ultraviolet-B radiation when applied to the skin of hairless mice 1 h before exposure.⁹⁾ EF 2001 also reduced the frequency of micronucleated reticulocytes and increased survival of sublethally irradiated mice without exhibiting estrogenic actions on reproductive systems.¹⁰⁾ The purpose of the tests reported here was to study *in vivo* radioprotection of EF 2001 on hematopoietic recovery contributing to increase survival of sublethally irradiated mice.¹¹⁾

Material and Methods

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.

Test material

Heat-treatment bacillus mort body (*Enterococcus faecalis* 2001; EF 2001), from EF 2001, lacking fungal products and designated EF 2001.

EF 2001[®], a bacillus product, composed of heat-treatment bacillus mort body, 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 6.5mg/g of the product. EF 2001[®] is hereafter described as β -glucan throughout this paper. EF 2001 was suspended in physiological saline to be concentrations of 2, 4, and 8% (w/v).

Radio-protective effect

Mice were oral medicated with EF 2001 suspended in physiological saline at a dose of 200 mg/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 2 Gy and 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.

Leukocyte and lymphocyte counts

Mice were oral medicated with EF 2001 suspended in physiological saline at a dose of 200 mg/kg and 400 mg/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).

NK activity

Mice were oral medicated with EF 2001 suspended in physiological saline at a dose of 200mg/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 ⁵¹C-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°C for 4 hr in a CO_2 -incubator. The radioactivity released into the supernatant was counted by a γ -counter, and the magnitude of cytotoxicity calculated based on the average radioactivity of the control group was defined as NK activity.

Statistical analysis

Significance of the difference in each parameter among groups was assessed by *t*-test and the Dunnett comparison test following analysis of variance. Values of $P < 0.05$ were considered significant.

Results

Survival rate of mice after irradiation

It followed from results that mortality increased markedly in all irradiated groups and most mice were dead within the 7-14 days following irradiation. At day 30 following irradiation, survival of irradiated

groups, by group, were 8 Gy group, 57.06%; 200 mg/kg + 8 Gy group, 77.25%; 400 mg/kg + 8 Gy group, 85.72%. It showed that, after EF 2001 pre-treatment, there was a significant enhancement in 8-day survival and about 80.61% higher than that of 400 mg/kg + 8 Gy group. The survival curve illustrated that, compared with the 8 Gy group data, the time to death was significantly shifted to the right for mice pre-treated with EF 2001 after irradiation. Those results demonstrated that EF 2001 possessed highly radioprotective efficacy on prevention of mortality in sublethally irradiated mice and its protecting actions were superior to that of EF 2001.

Leukocyte counts

The number of blood leukocytes in normal mice is summarized in Figs.1. The number of leukocytes increased with time at least up to 24 hr after each repeated dose of EF 2001 in a dose-dependent manner. Statistically significantly higher and increase of leukocyte counts in 200 mg/kg group were observed in comparison with control group. In addition, at

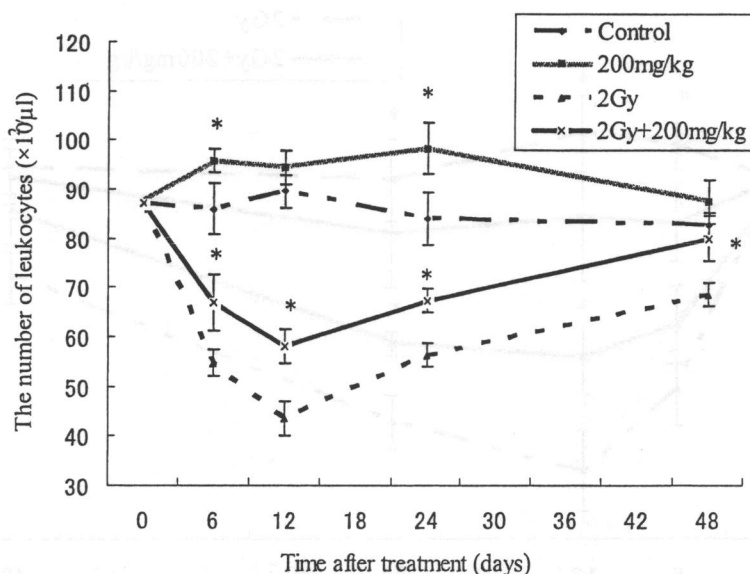


Fig.1. Leukocyte counts on different days after irradiation in mice of different groups.

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.

time of irradiation until day 12, statistically significantly higher and more rapidly recovery of leukocyte counts in 2 Gy + 200 mg/kg group were observed in comparison with 8 Gy group.

Lymphocyte counts

The number of blood lymphocytes in normal mice is summarized in Figs.2. The number of lymphocytes increased with time at least up to 24 hr after each repeated dose of EF 2001 in a dose-dependent manner. The lymphocyte counts also showed a similar tendency as in the leukocyte counts. Statistically significantly higher and increase of lymphocytes counts in 200 mg/kg group were observed in comparison with control group. In addition, at time of irradiation until day 12, statistically significantly higher and more rapidly recovery of lymphocytes counts in 2 Gy + 200 mg/kg group were observed in comparison with 8 Gy group.

NK activity

NK activity in mice is shown in Figs.3. Both of the NK activity increased significantly about twofold to threefold after each repeated dose of EF 2001 (200 and 400 mg/kg).

Mice were administrated with EF 2001 suspended in physiological saline at a dose of 200 or 400 mg/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 administration, spleen cells were prepared for measuring NK cell-mediated cytotoxicity by ^{51}Cr -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°C for 4 hr in a CO_2 -incubator. The radioactivity released into the supernatant was counted by a γ -counter, and the magnitude of cytotoxicity cal-

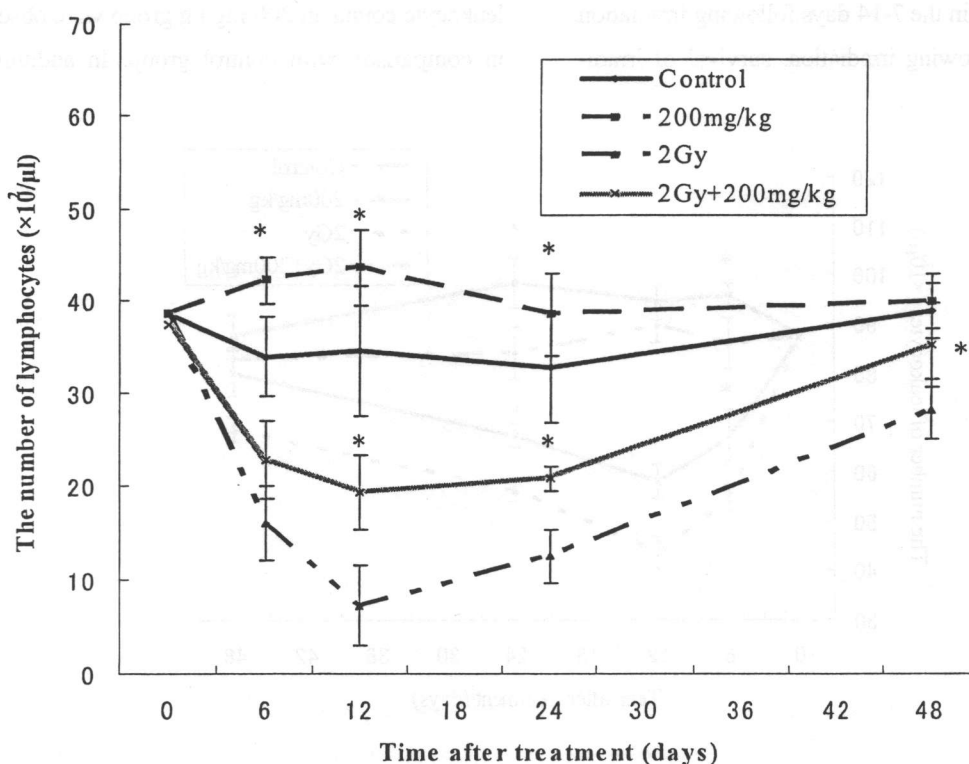


Fig.2. Lymphocyte counts on different days after irradiation in mice of different groups.

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.

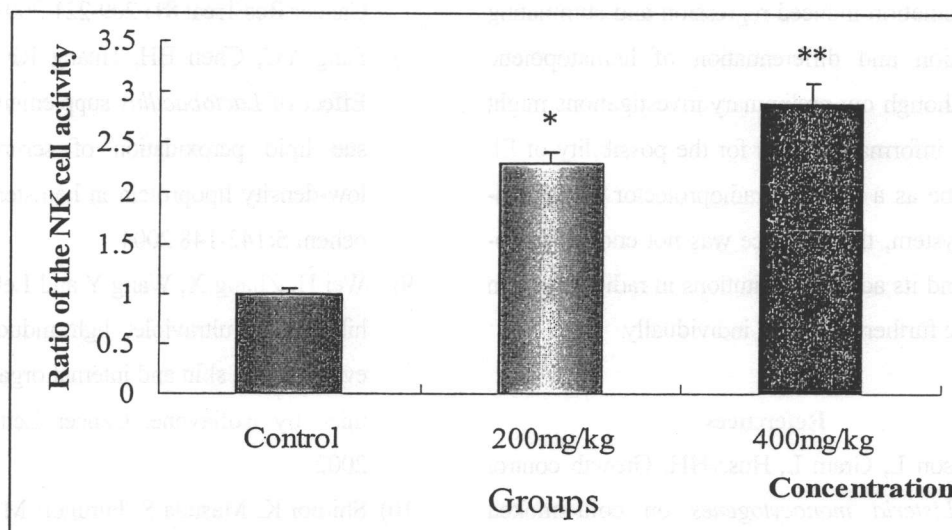


Fig.3. Repeated dose effect of EF 2001 on the NK activity in mice.

Groups of ten mice each were subjected to each treatment. Results represent means \pm S.D. * Statistically significant ($P < 0.05$) from the control group.

culated based on the average radioactivity of the control group was defined as NK activity.

Discussion

EF2001 is well known to exert radioprotective effect and anti-tumor effect *in vivo*¹²⁾ and these effects were reproduced in this study. To confirm the elucidative mechanisms by which EF 2001 these effects, the number of leukocyte and lymphocyte was monitored as a hemopoietic action. Furthermore, NK activity was measured as immunological parameters. The results of these parameters demonstrated that the radioprotective effect of EF 2001 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 EF 2001. In addition, augmented immunological activity as seen in increased NK activity by EF 2001 seems to play a role in preventing secondary infections associated with irradiation. Natural killer (NK) cells are well known to be associated with cytotoxic effect on various kinds of tumor cells.¹³⁾ Therefore, increased activity of NK by EF 2001 contributes probably to attenuated tumor growth in tumor-bearing mice. From these, EF 2001 is expected to be promising for the treatment of cancer patients

receiving radiotherapy. Accordingly, we used peripheral blood cell counts as indicators of bone marrow function in order to assess the radioprotection of normal tissue, which is critical for survival in this study. The data from our experiments showed that prior oral administrations of EF 2001 to mice with 200 mg/kg/day for consecutive 7 days rendered 80% survival in irradiated mice and its survival was significantly higher than that of irradiated control group as well as that of EF 2001 administration. Stimulating recovery of peripheral hematocytes were also observed in mice pre-treated with EF 2001, but protecting actions of EF 2001 on leukocytes and nucleated cells were more stronger than those of EF 2001, although its protection against the decrease of lymphocytes counts was lower than that of EF 2001. We inferred that EF 2001, like EF 2001, was an effective radioprotector against radiation-induced death by stimulating the rehabilitation of hematopoiesis. These properties have been associated preciously with radioprotection.¹⁴⁾

In summary, the results of the current study demonstrated that pre-treated with EF 2001 have some effects on promoting survival and accelerating the rehabilitation of hematopoiesis by protecting bone marrow stem cells and peripheral hematocytes

against radiation-induced regression and stimulating proliferation and differentiation of hematopoietic cells. Although our preliminary investigations might EF 2001 information basis for the possibility of EF 2001 to be as a selective radioprotector of hematopoietic system, the evidence was not enough to apply yet and its active constitutions in radioprotection should be further examined individually.

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