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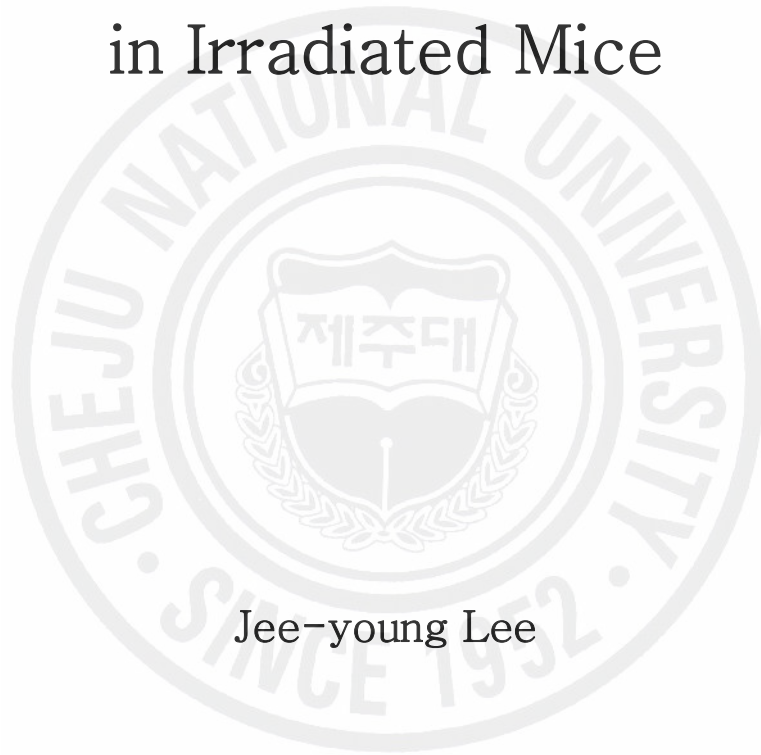
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A Thesis

For the Degree of Master of Veterinary Medicine

Effects of Fucoidan on Survival
and Hematopoietic Recovery
in Irradiated Mice



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Effects of Fucoidan on Survival and Hematopoietic Recovery in Irradiated Mice

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2006. 10.

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Abstract

To evaluate the possible *in vivo* radioprotection conferred by fucoidan, the survival rate and hematopoietic changes in mice subjected to total body irradiation were investigated. BALB/c mice were administered 10, 50, or 100 mg of fucoidan/kg body wt i.p. before exposure to 9 Gy of gamma radiation, and then were monitored for 30 days. To see the effect of fucoidan on the radiation-induced damage to peripheral blood leukocytes, the number of blood leukocytes was counted in mice treated with fucoidan 4 h post-irradiation (6 Gy). The changes in bone marrow viability and the endogenous hematopoietic spleen colony formation after irradiation (6 Gy) were measured at 9 days in mice given fucoidan (10 and 100 mg/kg body wt, i.p.). The fucoidan pretreatment improved the survival rate of irradiated mice; 67% (6/9) of the mice treated with 100 mg/kg of fucoidan survived until day 30 after exposure to 9 Gy of gamma radiation, whereas most of the mice treated with less fucoidan died before day 15. This effect accompanied significant increases in peripheral blood leukocytes, endogenous spleen colonies, and bone marrow cells in irradiated mice pretreated with fucoidan, compared with control mice. Pretreatment with fucoidan before irradiation plays an important role in the survival of irradiated mice, possibly by protecting hematopoietic cells, including peripheral blood leukocytes, bone marrow cells, and

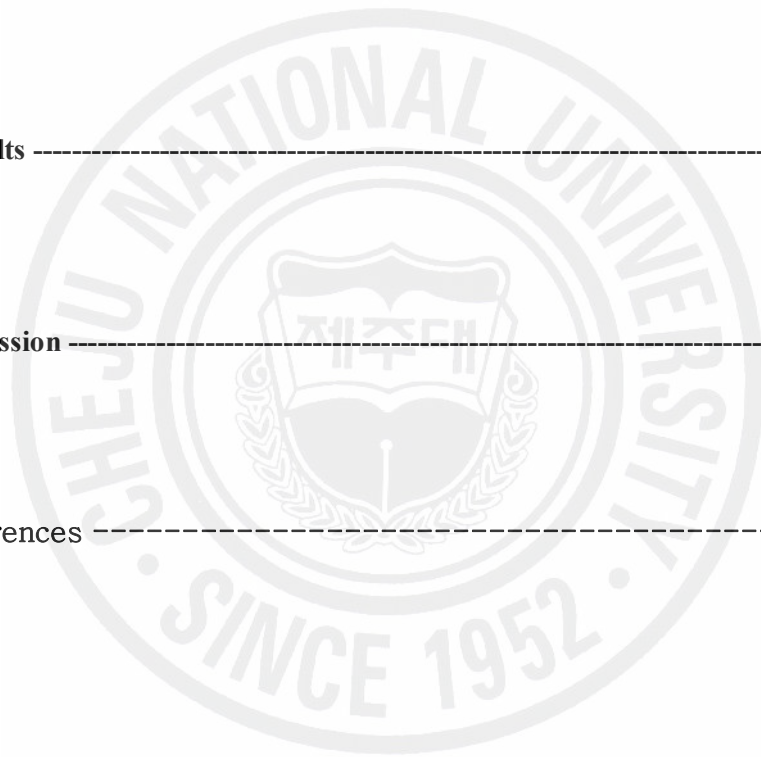
endogenous spleen colonies. The molecular mechanism of the radioprotection conferred by fucoidan remains to be determined.

Keywords: Fucoidan, Irradiation, Spleen, Bone marrow cells



CONTENTS

I. Introduction	1
II. Materials and Methods	3
III. Results	6
IV. Discussion	14
V. References	16



I. Introduction

Radiation is used to treat various malignancies [4]. However, a severe side effect of radiotherapy is that it damages normal cells. The hematopoietic system and hematocytes are sensitive to low doses of radiation [14]. Radioprotective agents are administered before exposure to ionizing radiation to reduce the damaging effects, including radiation-induced lethality [14]. The efficacy of many synthetic and natural agents at protecting against radiation injuries has been investigated in recent years [9].

Fucoidans are a group of sulfated fucose-containing polysaccharides that are derived from marine brown algae including *Fucus vesiculosus* [1]. Many studies have shown that fucoidans have a wide spectrum of activity in biological systems [10]. Fucoidans have potent anti-thrombotic activity [1]. Another outstanding physiological function of fucoidans is their anti-inflammatory properties via anti-complement [2] and anti-leukocyte migration effects [7].

In vivo, fucoidan blocks leukocyte rolling in a dose-dependent manner, interfering with various inflammatory responses in several animal models [3, 6, 11]. In addition, it was reported that fucoidan increased circulating mature white blood cells and hematopoietic

progenitor/stem cells in mice and nonhuman primates [11].

Little is known of the biological effects of fucoidan *in vivo* in animals that have been irradiated. Therefore, this study investigated the effects of fucoidan on mice given a sublethal dose of radiation.



II. Materials and Methods

1. Animals and experiments

Sixty female BALB/c mice (Orient Bio, Gyunggi-do, Korea), 6 to 8 weeks old, were used in this experiment. Fucoidan (Sigma, St. Louis, MO, USA) was dissolved in phosphate-buffered saline (PBS) and administered intraperitoneally (i.p.) 24 and 1 h before irradiation. Control animals were given PBS instead, at the same times.

After treatment, the mice were placed in a specially designed, well-ventilated acrylic container and subjected to whole-body radiation with 6 or 9 Gy in a single fraction using a ^{60}Co γ -ray source (10,000 Ci; Co-60 Irradiation Facility, Applied Radiological Science Research Institute, Cheju National University, Korea).

2. Survival assays

Survival was monitored daily and reported as the percentage of animals surviving 30 days after 9 Gy irradiation. Each treatment group (irradiation plus vehicle or 10, 50, or 100 mg fucoidan /kg body weight) consisted of nine mice. The data are expressed as the percent survival.

3. Hematologic examination

Four hours following irradiation (6 Gy), 1 ml of blood was collected from the hearts of mice treated with nothing (n = 3), fucoidan only (n = 3), irradiation only (n = 3), and irradiation plus fucoidan (100 mg/kg body weight) (n = 3) into tubes containing ethylenediaminetetraacetic acid (EDTA) before sacrifice. Total leukocytes were counted microscopically with a hemocytometer.

4. Hematopoietic stem cell assays

To test the effect of fucoidan after irradiation, the number of bone marrow cells was counted 9 days after 6 Gy irradiation. Each treatment group (normal control plus vehicle, irradiation plus vehicle, and irradiation plus fucoidan 10 or 100 mg/kg) consisted of five mice. Bone marrow cells were obtained from anesthetized mice by aseptic isolation of the femurs from which the marrow was flushed with Hank's balanced salt solution (HBSS) using a 25-gauge needle. The cells were suspended in HBSS and counted with a hemocytometer. The results are expressed as the number of live bone marrow cells per 10^6 cells/femur.

To test the effect of fucoidan on endogenous hematopoietic spleen colony formation, the number of spleen colonies was counted using the method of Till and McCulloch [12] on

day 9 after radiation. The mice were sacrificed; their spleens were removed and fixed in Bouin's fixative for 24 h. The number of macroscopic spleen colonies was then counted.

5. Histological examination

For histological examination, femurs (n=5/each group) from mice (normal control plus vehicle, irradiation plus vehicle, and irradiation plus fucoidan 10 or 100 mg/kg) were isolated and fixed in 4% paraformaldehyde in phosphate buffer, decalcified in the decalcification solution (formic acid-sodium citrate method) [8] for five days and processed for paraffin embedding. Paraffin sections were stained with hematoxylin and eosin.

6. Statistical analysis

The results are presented as the mean \pm S.E. The results were analyzed statistically using one-way analysis of variance (ANOVA) followed by the *post-hoc* Student-Newman-Keuls procedure for multiple comparisons. In all cases, $p < 0.05$ was considered significant.

III. Results

1. Survival rate of mice after irradiation

Of the control mice given 9 Gy irradiation, 78% (7/9) died by day 10, and all died before 15 days post-irradiation (Fig. 1). The mortality of the irradiated mice pretreated with fucoidan 10 mg/kg was 67% (6/9) at day 10, and 100% by day 15. The mortality of the mice pretreated with fucoidan 50 mg/kg was delayed: 56% (5/9) died at day 10, and the remainder (4/9) all died by day 15. The mortality of the irradiated mice treated with 100 mg fucoidan/kg was significantly reduced compared with that of the other groups. Only 22% (2/9) of the mice died before day 15, and 67% (6/9) remained alive at day 30. These results suggest that fucoidan decreases the radiation-induced mortality.

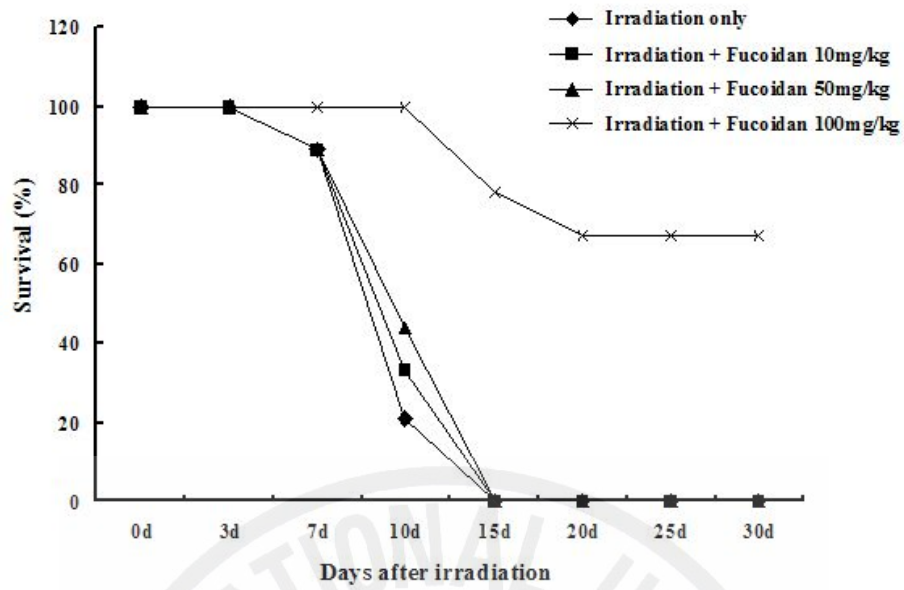


Fig. 1. Effect of different doses of fucoidan on the survival of mice exposed to 9 Gy irradiation. The data are expressed as the percent survival.

2. Hematologic examination

To see the effect of fucoidan on the radiation-induced damage to peripheral blood leukocytes, the blood leukocytes were counted in untreated mice and in mice treated with irradiation only, fucoidan only, and irradiation plus fucoidan (100 mg/kg).

Total body radiation significantly lowered the total leukocyte count (Fig. 2). The number of peripheral blood leukocytes in the irradiation-only group ($2,483 \pm 324/\text{mL}$) was significantly lower than in the fucoidan-only ($7,367 \pm 1024/\text{mL}$) and normal control ($7,500 \pm 425/\text{mL}$) groups (both $p < 0.01$). The peripheral blood leukocytes in the mice treated with irradiation plus 100 mg/kg of fucoidan were significantly increased compared with the number in the irradiation-only group ($4,617 \pm 451/\text{mL}$ vs. $2,483 \pm 324/\text{mL}$; $p < 0.05$).

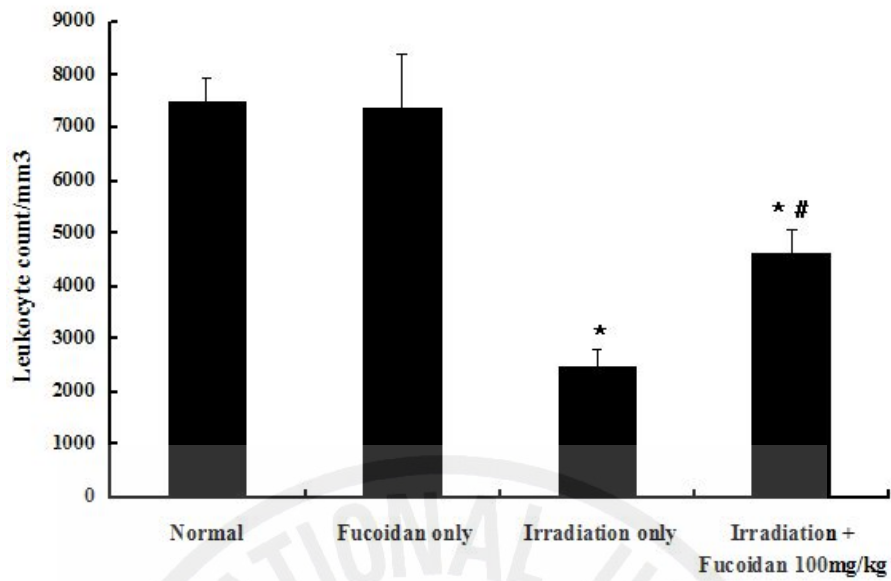


Fig. 2. Effect of the administration of fucoidan on the total leukocyte count of irradiated animals. Irradiation lowered the total leukocyte count, and the administration of fucoidan elevated the levels. The data are expressed as the mean \pm S.E. * $p < 0.05$ compared with the normal controls; # $p < 0.05$ compared with the irradiation-only group.

3. Effect of fucoidan on bone marrow nucleated cells

Bone marrow viability (Fig. 3) was significantly lower in the irradiation-only group ($4.46 \pm 0.79 \times 10^6$ cells/femur) than in the normal control group ($16.88 \pm 0.06 \times 10^6$ cells/femur, $p < 0.001$). In the irradiation plus 100 mg fucoidan/kg group, bone marrow viability was significantly higher compared with that in the irradiation-only group ($8.36 \pm 0.59 \times 10^6$ vs. $4.46 \pm 0.79 \times 10^6$ cells/femur, $p < 0.001$). However, there was no significant difference in bone marrow viability between the irradiation-only and irradiation plus 10 mg fucoidan/kg groups ($4.46 \pm 0.79 \times 10^6$ vs. $2.65 \pm 0.94 \times 10^6$ cells/femur, $p > 0.05$).

Histological examination of bone marrow from irradiated mice showed marked hypocellularity (Fig. 4B) in comparison to normal cellularity in untreated control mice (Fig. 4A). Fucoidan (100 mg/kg) administration elevated the lowered cellularity of bone marrow after irradiation (Fig. 4D). This suggests that the higher dose of fucoidan (100 mg/kg) effectively protects the bone marrow cells of mice exposed to radiation.

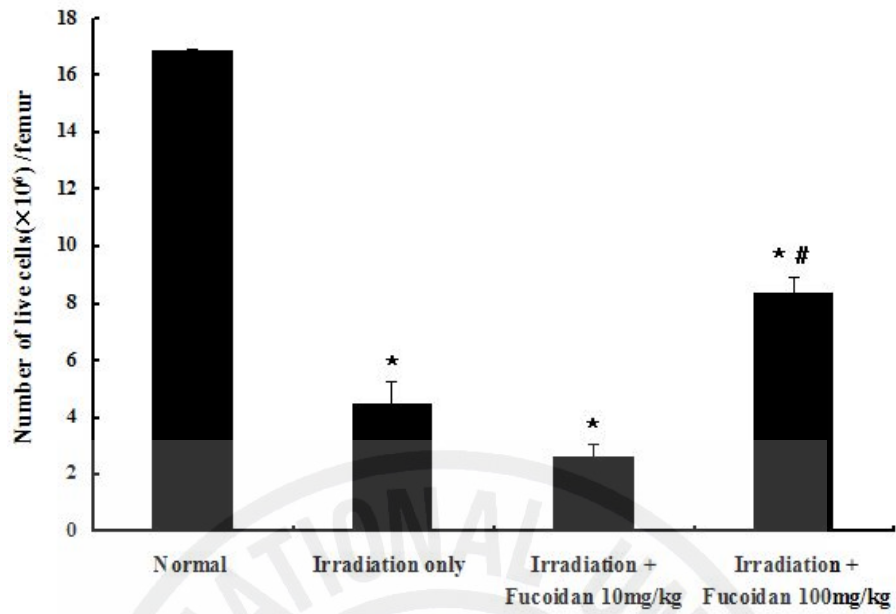


Fig. 3. Effect of the administration of fucoidan on the bone marrow cellularity of radiation-treated animals. Fucoidan treatment increased the bone marrow cellularity. The data are expressed as the mean \pm S.E. * $p < 0.05$ compared with the normal controls; # $p < 0.05$ compared with the irradiation-only group.

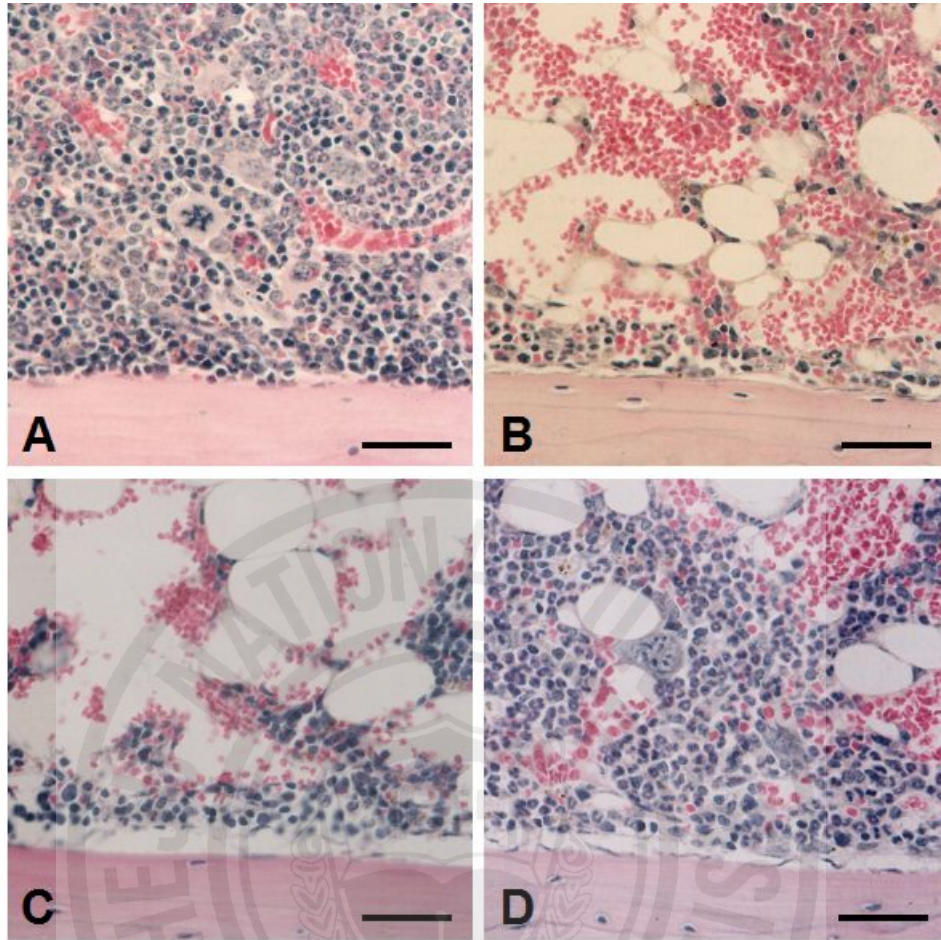


Fig. 4. Histological findings in bone marrow of the normal control (A), irradiation-only (B), irradiation plus 10 mg fucoidan/kg (C), and irradiation plus 100 mg fucoidan/kg groups (D).

The scale bars represent 40 μm . Panels A–D stained with hematoxylin-eosin.

4. Effect of fucoidan on endogenous hematopoietic spleen colony formation

The results for spleen colonies (Fig. 5) largely matched those for bone marrow cells.

Few spleen colonies were found in the spleens of mice given sub-lethal irradiation. The

number of spleen colonies was increased significantly in the mice that received 10

(7.8 ± 0.37) or 100 (15.25 ± 0.48) mg fucoidan/kg compared with the mice exposed to

irradiation only (2.2 ± 0.37) and the normal controls (1.5 ± 0.5) ($p < 0.001$).

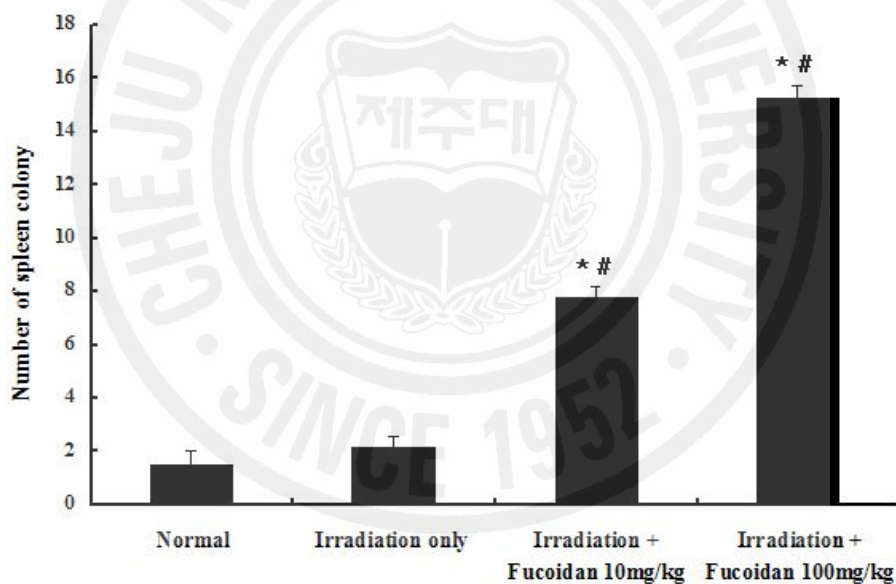


Fig. 5. Effect of the administration of fucoidan on endogenous hematopoietic spleen colony

formation in irradiated animals. The data are expressed as the mean \pm S.E. * $p < 0.05$

compared with the normal controls; # $p < 0.05$ compared with the irradiation-only group.

IV. Discussion

Our study indicates that fucoidan at a dose of 100 mg/kg provided the highest protection against radiation-induced mortality. Moreover, our data show that the pre-irradiation administration of fucoidan stimulated the recovery of total leukocytes and reduced the decrease in bone marrow nucleated cells induced by radiation. Therefore, fucoidan administration appears to protect both circulating blood cells and progenitor cells from irradiation. The enhanced spleen colony counts in the fucoidan-treated irradiated mice indicates that fucoidan protects stem cells or stimulates the proliferation of surviving cells. These results suggest that the increase in the 30-day survival is attributable to the protection afforded by fucoidan to the stem cell compartments of the bone marrow and spleen, which continue to supply the requisite number of cells in survivors.

It was reported that fucoidan elevated circulating white blood cells and mobilized hematopoietic progenitor/stem cells within hours [11]. The cells mobilized by fucoidan included radioprotective cells and probably also the cells required for long-term repopulation [11]. Therefore, the protective effect on leukocytes and bone marrow cells after irradiation seen in our study is supported by these roles of fucoidan.

Fucoidan is a ligand for the macrophage scavenging receptor 1 (MSR1), which increases the level of tumor necrosis factor- α and interleukin-1 secretion [5]. It was recently reported that fucoidan inhibited the lipopolysaccharide-mediated expression of inducible nitric oxide synthase by blocking activator protein 1 activation in macrophages [13]. Recently, fucoidan was proposed to prevent concanavalin A-induced liver injury through the production of endogenous interleukin-10 by Kupffer cells, and these effects were mediated by MSR1 [10]. These anti-inflammatory roles of fucoidan may be another important mechanism of its radioprotective efficacy.

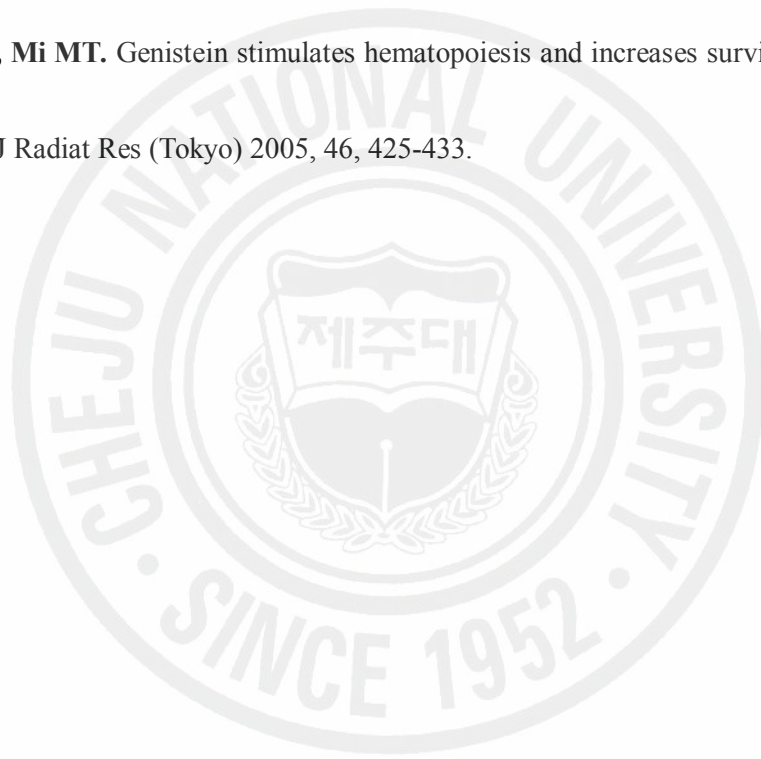
Taken all into considerations, we postulated that fucoidan administration before irradiation has a dose-dependent effect on increasing the survival of irradiated mice by stimulating hematopoiesis. The results suggest that fucoidan is useful for radioprotection.

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제가 수의학이란 학문을 접하기 시작하여 지금까지 저에게 많은 가르침을 주신 김희석 교수님, 박전홍 교수님, 배종희 교수님, 이경갑 교수님, 정종태 교수님, 임윤규 교수님, 이두식 교수님, 이영재 교수님, 우호춘 교수님, 손원근 교수님, 이주명 교수님, 강태영 교수님, 윤영민 교수님, 김재훈 교수님, 황규계 교수님, 박현정 교수님, 그리고 고인이 되신 이국경 교수님께도 감사의 마음을 전합니다.

학부때 실험실에 들어와서부터 대학원까지 많은 도움을 주신 이용덕 선생님, 김황룡 선배님, 문창중 교수님, 안미정 선배님 그리고 대학원 생활 내내 곁에서 조언과 도움을 주신 김희철 선배님, 실험실에서 저를 많이 도와준 태기, 찬우, 지성, 정태오빠, 진우에게도 감사드립니다. 저와 같이 대학원에 들어와 서로 힘이 되어준 원형오빠, 현호오빠, 보람오빠, 민희, 미형에게도 고마운 마음을 전합니다.

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