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Protective Effects of *Phellinus linteus* Polysaccharides Against Radiation-Induced Hematopoietic Damage in Bone Marrow of Mice

Hye-Song Kim*, Mok-Ran Jon, Won-Song Paek

Abstract

Chemotherapy and radiation therapy, which are standard methods of treatment for various cancers, often result in suppression of hematopoiesis accompanied by reduction in the number of hematopoietic stem cells and their progeny. We investigated a clinically potential use of Phellinus linteus polysaccharide in the treatment of various cytopenias induced by radiotherapy. Mice were administered Phellinus linteus polysaccharide orally at a dosage of 100 mg/kg once daily for + consecutive days, either prior to (pre-treatment) or following gamma-irradiation (therapy). Those receiving pre-treatment exhibited reduced sensitivity to radiation. After 10 days following irradiation, there was a decline in the levels of red blood cells, white blood cells, and platelets. Nonetheless, the count in the group pre-treated with Phellinus linteus polysaccharide was elevated in comparison to the control group. All blood cells increased significantly as compared to control ($P<0.05$) after 20 days of irradiation. The number of bone marrow cells and spleen in mice treated with Phellinus linteus polysaccharide had significantly increased after radiation as compared to control ($P<0.05$). But there was no significant difference between the number of bone marrow cells and spleen in mice of III and IV groups treated with Phellinus linteus polysaccharide, and blank control ($P>0.05$). The results of the present experimental study showed that Phellinus linteus polysaccharide can accelerate restoration of radiation-induced hematopoietic damage in bone marrow and spleen of mice. Additional research is required before considering its application in safeguarding humans against ionizing radiation, particularly in the context of treating cancer patients undergoing chemotherapy and radiotherapy.

Keywords: chemotherapy, radiation therapy, hematopoietic stem cells, *Phellinus linteus* polysaccharide, Gamma-irradiation, red blood cells, cancer treatment

INTRODUCTION

Standard methods of treatment for various cancers consist of chemotherapy and radiation therapy, and especially the importance of radiotherapy is increasingly recognized [1, 2]. These treatments often result in suppression of hematopoiesis accompanied by reduction in the number of hematopoietic stem cells and their progeny [3]—a primary cause of immunodeficiency and death [4].

Ionizing radiation is well known as the potential oncogenic agent. Exposure of living cells to ionizing radiation induces a multifaceted and dose-dependent sequence of physiological and morphological changes, referred to as hematopoietic syndrome, which can potentially be fatal [5, 6].

Recently many studies have been conducted to find various natural products with radioprotective effects.

***Author for Correspondence**
Hye-Song Kim
E-mail: pmed10@ryongnamsan.edu.kp

Researcher, Department of Hematology, Pyongyang University of Medical Sciences, Pyongyang, Democratic People's Republic of Korea

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Mounting clinical, toxicological, and biochemical evidence suggests the utilization of various natural products as supplementary therapies for patients undergoing radiation exposure and in strategies aimed at preventing cancer development [7–9].

Efforts to discover effective and safe compounds with radiation protection properties have sparked growing interest in naturally occurring medicinal plants. Mushrooms are renowned as dietary supplements believed to offer health benefits. *Phellinus linteus*, a dark parasitic fungus, thrives on the living trunks of mulberry trees [10, 11].

It is also known as 'sanghuang' and was traditionally used as an effective folk medicine for a long time [12]. *P. linteus* plays a significant role in health problems. It has been used in the treatment of allergies, diabetes, hyperlipemia, and liver fibrosis for its medicinal effects and it shows the potential for development of anticancer therapy [13–16].

This function has been credited to the biological effectiveness of its diverse elements, including polysaccharides, triterpenoids, polyphenols, and pyrans. Polysaccharides constitute a significant portion of *P.linteus* and have demonstrated various biological properties, such as anti-inflammatory, anti-tumor, antioxidant, hypoglycemic, and immune-stimulating effects [17–23].

Many recent reports have demonstrated the health-promoting functions of *P.linteus* including protection of DNA from the damage induced by oxidative stress, anti-inflammatory and anti-nociceptive effects [24].

Moreover, β-Glucans belonging to a group of polysaccharides isolated from *P. linteus* have been demonstrated to enhance the production of hematopoietic progenitors' cells in normal and gamma-irradiated mice [25].

These findings indicated that *P. linteus* has multiple applications in medicinal treatments. In the present study, we assessed the protective effect of *P.linteus* polysaccharide (PLP) on recovery from the radiation-induced hematopoietic damage in mice.

MATERIAL AND METHODS

Animals

Male and female mice (6–8 weeks old, weighing 18–22 g) were used for the present study. The animals were kept in an environment with a room temperature of (22±2) °C and provided with a standard laboratory rodent diet.

Irradiation

The recipient mice were whole-body irradiated with a single dose 4Gy using a ^{60}Coγ-ray (X-ray) source.

Preparation of Polysaccharides from *Phellinus linteus*

P. linteus was collected locally, identified, air dried, and powdered. The powder *of P.linteus* was subjected to three rounds of water extraction at 60 °C for 4 h each. Following extraction, the resulting solution was centrifuged at 4000 revolutions per minute for 20 min, and the supernatant was combined, dialyzed using a Spectra/Por RC dialysis membrane with a molecular weight cutoff of 3500 Da, and subsequently concentrated. To precipitate the polysaccharides, three volumes of 95% ethanol were added and left at 4 °C overnight. The resulting precipitate was collected through centrifugation at 4000 revolutions per minute for 10 min, washed with absolute acetone, and then air-dried at 35 °C to obtain the water-soluble polysaccharide.

Experimental Design

The mice were randomly distributed into four groups:

1. Animal of Group-I: They were administrated saline solution for 20 consecutive days to serve as blank control.

2. Animal of Group-II: They were administrated saline solution for 20 consecutive days after irradiation to serve as treatment control.
3. Animal of Group-III: They were administrated PLP orally 100 mg/kg once a day for 20 days to serve as prophylaxis and then irradiated.
4. Animal of Group-IV: They were administrated PLP orally 100 mg/kg once a day for 20 days after irradiation to serve as experimental.

All (control and treatment) groups included ten mice each.

Blood Cell Counts

Blood samples were drawn from the tail vein every 10 days and 20 days apart from the initiation of the experiment. The samples were processed for analysis immediately after collection. The number of erythrocytes, leukocytes and thrombocytes were counted using a haemocytometer.

Preparation of Bone Marrow Smears and Cellular Counting

The femurs were extracted from autopsied mice and trimmed on both sides.

- Bone marrows were drawn out on the slide by syringing through 20-gauge needle.
- Bone marrows were diluted with saline solution of 1 ml and pipetted with blood pipe to 0.5 mark and then diluted white blood dilute fluid to the eleven marks.
- Bone marrow cells were counted by method of white blood cell determination.

Spleen Hematopoietic Colonies

Mice were killed and their spleens were removed and placed in Bouam solution for 30 min. Then the hematopoietic colonies on the surface of the spleen were counted under a dissecting microscope.

Statistical Analysis

The results were presented as mean±standard error (SE). A t-test was employed to conduct statistical comparisons between the groups. Significance levels were set as $P<0.05$.

RESULTS

Effect of PLP on Hematopoietic Recovery

Peripheral blood cell counts were examined after irradiation to estimate the effect of PLP on hematopoietic recovery (Table 1). Ten days post-irradiation, there was a decrease in the count of red blood cells (RBCs), white blood cells (WBCs), and platelets. However, the count in the PLP pretreated group was greater as compared to the control. Twenty days after irradiation, all blood cells increased significantly as compared to control ($P<0.05$). These results showed that mice fed with PLP were less sensitive to the adverse effects of whole-body irradiation.

Table 1. Variation of peripheral blood counts pre-and post-irradiation M±SE.

Parameter	Group	Pre-irradiated	Post-irradiated	
			10 days	*20 days*
Red blood cells ($\times10^{12}$/L)	I	7.68±0.72	7.72±0.79*	7.75±0.71*
	II	7.75±0.71	3.57±0.12	4.25±0.24
	III	7.72±0.81	5.75±0.23*	6.35±0.26*
	IV	7.69±0.75	3.98±0.17	5.76±0.25*
White blood cells ($\times10^{9}$/L)	I	10.75±0.28	10.72±0.27*	10.77±0.26*
	II	10.68±0.25	4.27±0.23	5.17±0.31
	III	10.72±0.27	7.93±0.22*	8.85±0.25*
	IV	10.69±0.24	5.24±0.23	7.95±0.27*
Platelets	I	425.3±14.77	423.2±14.5*	427.6±14.6*

($\times 10^9$/L)	II	423.3±14.8	265.7±12.4	269.5±13.1
	III	427.7±14.5	342.3±13.6*	388.8±13.9*
	IV	428.4±15.1	288.7±12.8	356.4±13.8*

**; P<0.05 (compared with group II)*

Effect of PLP on Bone Marrow Cellularity

The number of bone marrow cells in mice treated with PLP had significantly increased after radiation as compared to treatment control (P<0.05) as shown in Table 2. But there was no significant difference between the number of bone marrow cells in mice of III and IV groups treated with PLP and blank control (P>0.05).

Table 2. Effect of PLP on bone marrow cellularity (M±SE).

Group	Number of BM cells ($\times 10^5$/leg)
I	43.80±1.36*
II	29.60±1.25
III	42.20±1.32*
IV	41.50±1.29*

**P<0.05 (compared with group II)*

Effect of PLP on Spleen Hematopoietic Colonies

Table 3 shows that the spleen hematopoietic colonies in mice of III and IV groups treated with PLP was increased markedly than in treatment control (P<0.05). There was no significant difference between the spleen hematopoietic colonies in mice of III and IV groups treated with PLP, and blank control (P>0.05).

Table 3. Effect of PLP on spleen hematopoietic colonies (M±SE).

Group	Number of hematopoietic colonies (nodules/spleen)
I	29.25±1.92*
II	20.71±1.88
III	28.54±1.76*
IV	27.43±1.62*

**P<0.05 (compared with group II)*

DISCUSSION

The main purpose of the present study was to investigate that PLP possess protective effect against radiation-induced hematopoietic damage in mice. Mice used in the present study were exposed to dose of 4 Gy, because comparable results with 9 Gy permitted us to investigate a dose which corresponds more to a real therapeutic dose for humans.

There is substantial and consistent evidence for radioprotective effect of various kinds of mushrooms but the data from the PLP are still limited. *P. linteus* has been used in the treatment of allergies, diabetes, hyperlipidemia, and liver fibrosis for its medicinal effects and it shows the potential for development of anticancer therapy [13–16].

It has been suggested that the therapeutic activities of *P.linteus* depend mainly on the presence of PLP [12, 13]. Polysaccharides isolated from *P. linteus* have been demonstrated to exhibit many biological activities including anti-inflammatory, antitumor [17, 18], antioxidant [19, 20], hypoglycemic [21], immune-stimulating [22, 23], antimutagenic, and antimetastatic effects. Many recent reports have demonstrated the health-promoting functions of *P.linteus* including protection of DNA from the damage induced by oxidative stress, anti-inflammatory, and antinociceptive effects [24, 25].

Moreover, β-Glucans belong to a group of polysaccharides isolated from *P. linteus* have been demonstrated to enhance the production of hematopoietic progenitors' cells in normal and gamma-irradiated mice.

The number of RBCs, WBCs, and platelets decreased after ten days of irradiation; but the number in PLP-pretreated group was higher than treatment control. All blood cells increased significantly after 20 days of irradiation as compared to treatment control ($P<0.05$).

These results showed that mice fed with PLP were less sensitive to the adverse effects of whole-body irradiation. It is well known that the time of administration of radioprotective agents is critical for both diminishing hematopoietic damage and increasing the rate of survival.

Administration of PLP for 20 days before irradiation showed a significant increase in number of whole blood cells. The number of bone marrow cells in mice treated with PLP had significantly increased after radiation as compared to treatment control ($P<0.05$).

But there was no significant difference between the number of bone marrow cells in mice of III and IV groups treated with PLP and blank control ($P>0.05$). The spleen hematopoietic colonies in mice of III and IV groups treated with PLP was increased markedly than in treatment control ($P<0.05$).

The results demonstrated that administration of PLP prior to and after ionizing irradiation in mice significantly promoted recovery of hematopoiesis, as indicated by bone marrow and spleen hematopoietic colonies.

CONCLUSION

Results of the present experimental study showed that PLP can accelerate restoration of radiation-induced hematopoietic damage in mice.

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