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Protective effects of pellinus linteus polysaccharides against radiation induced hematopoietic damage in bone marrow of mice

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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 (PLP) in the treatment of various cytopenias induced by radiotherapy. The mice were given Phellinus linteus polysaccharide orally 100 mg/kg once a day for twenty consecutive days either before (pre-treatment) or after gamma-irradiation (therapy). Mice that received pre-treatment were less sensitive to irradiation. Ten days after irradiation, the number of Red Blood Cells (RBC), White Blood Cells (WBC) and platelets decreased, but the number in PLP pre-treated group was higher than control. Twenty days after irradiation, all blood cells increased significan tly compared to control ($P<0.05$). The number of bone marrow cells and spleen in mice treated with PLP had significantly increased after radiation 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 PLP,and blank control ($P>0.05$). Our experimental results show that Phellinus Linteus Polysaccha ride (PLP) can accelerate restoration of radiation induced hemato poietic damage in bone marrow and spleen of mice. However, further research is necessary before implementation in human protection against ionizing radiation, especially in the treat ment of cancer patients exposed to chemotherapy and radiotherapy.

Introduction

Standard methods of treatment for various cancers are consists of chemotherapy and radiation therapy, and especially the importance of radiotherapy are 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 oncogen.

Exposure of living cells to ionizing radiation causes the development of a complex, dose dependent series of potentially fatal physiologic and morphologic changes, known as hematopoietic syndrome [5,6].

Recently many studies have been carried out to found the various natural products with the radioprotective effects.

Growing clinical, toxicological and biochemical evidence supports the use of different natural products as adjunct treatment for pati ents exposed to radiation as well as in chemo preventive strategies [7-9].

Investigations for effective and non-toxic compounds with radio protection capability led to increasing interest in naturally growing medical plants.

Mushrooms are well known as nutritional supplements that are claimed to be beneficial for human health.

Phellinus linteus is a black parasitic fungus that grows on the living trunks of mulberry tree [10,11].

It is also known as'sanghuang'and was traditionally used as an effective folk medicine for a long time [12].

Phellinus linteus plays a significant role for health problems.

Phellinus linteus 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 role has been attributed to the biological activity of its various components such as polysaccharides, triterpenoids, polyphenols and pyrans.

Polysaccharides are one of the main components of Phellinus linteus and they have been shown to exhibit many biological activities including anti-inflammatory, anti-tumor [17,18], antioxidant [19,20], hypoglycemic [21], immune stimulating effects [22,23].

Many recent reports have demonstrated the health-promoting functions of Phellinus linteus including protection of DNA from the damage induced by oxidative stress, anti-inflammatory and anti-nociceptive effects [24-27].

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 [28].

These findings indicate that P. linteus has multiple applications in medicinal treatments.

In the present study, we assessed the protective effect of Phellinus 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 from 18 g to 22 g) were used for the present study.

The animals were maintained at a room temperature (22±2) °C and fed a standard diet for laboratory rodents.

Irradiation

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

Preparation of Polysaccharides from Phellinus linteus Phellinus linteus was collected locally, identified and were air dried, powdered.

The powder of Phellinus linteus was extracted three times with water at 60°C for 4 h. The extract was centrifuged at 4000 r/min for 20 min, and the supernatant was combined, dialyzed (Spectra/Por RC dialysis membrane, MW cutoff 3500 Da), and then concentrated. Three volumes of 95% ethanol were added to preci pitate polysa ccharides at 4°C overnight. The precipitate was collected after centrifugation at 4000 r/min for 10 min, washed with absolute acetone and then air dried (35°C) to yield the water soluble polysaccharide.

Experimental design

The mice were randomly distributed into four groups.

Animals of group-I were administrated saline solution for twenty consecutive days to serve as blank control.

Animals of group-II were administrated saline solution for twenty consecutive days after irradiation to serve as treatment control.

Animals of group-III were administrated PLP orally 100 mg/kg once a day for 20 days to serve as prophylaxis and then irradiated.

Animals of group-IV were administrated PLP orally 100 mg/kg once a day for 20 days after irradiation to serve as experimental.

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

Blood cell counts

From the initiation of the experiment every 10 days and 20 days apart, blood samples were drawn from the tail vein and processed for analysis immediacy after collection.

The number of erythrocyte, leukocyte and thrombocyte were counted using a haemocytometer.

Bone marrow smears preparation and cellular counting

Femurs were dissected out from autopsied mice and catted off the both sides.

Bone marrow were drawn out on the slide by syringing through 20 gauge needle.

Bone marrow 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 mark.

Bone marrow cells were counted by method of white blood cell determination.

Spleen hematopoietic colonies

Mice were killed their spleens 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 obtained were expressed as mean ± SE.

T- test was used to make a statistical comparison between the groups.

Significance levels were set $P<0.05$.

Results

Effect of PLP on hematopoietic recovery

To estimate the effect of PLP on hematopoietic recovery, we examined peripheral blood cell counts after irradiation.

Ten days after irradiation, the number of Red Blood Cells (RBC), White Blood Cells (WBC) and platelets decreased, but the number in PLP pre-treated group was higher than control.

Twenty days after irradiation, all blood cells increased significantly compared to control (P<0.05).

These results shows 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*
Platelet ($\times10^{9}$/L)	I	425.3±14.77	423.2±14.5*	427.6±14.6*
	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)

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

Group	Number of BMcells ($\times10^{5}$/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)

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)

Effect of PLP on bone marrow cellularity

As shown in Table 2, the number of bone marrow cells in mice treated with PLP had significantly increased after radiation 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).

Effect of PLP on spleen hematopoietic colonies

As shown in Table 3, 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).

Discussion

The main purpose of the present study is to investigate that Phellinus Linteus Polysaccharide (PLP) possess protective effect of against radiation induced hematopoietic damage in mice.

Mice used in our study were exposed to dose of 4 Gy, because similar results with 9 Gy permitted us to investigate a dose which corresponds more to a real therapeutic dose for humans.

There is a substantial and consistent evidence for radio protective effect of various kinds of mushrooms but the data from the Phellinus Linteus Polysaccharide (PLP) are still limited.

Phellinus linteus 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].

It has been suggested that the therapeutic activities of Phellinus linteus depend mainly on the presence of polysaccharide (PLP) [12,13].

Polysaccharides isolated from P. linteus have been demonstrated to exhibit many biological activities including anti-inflammatory, anti-tumor [17,18], antioxidant [19,20], hypoglycemic [21] and immune stimulating effects [22,23] and and antimutagenic, antimetastastic effects.

Many recent reports have demonstrated the health-promoting functions of Phellinus 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.

In our experiment, ten days after irradiation, the number of Red Blood Cells (RBC), White Blood Cells (WBC) and platelets decreased, but the number in PLP pre-treated group was higher than treatment control.

Twenty days after irradiation, all blood cells increased significantly compared to treatment control (P<0.05).

These results shows 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 Phellinus Linteus Polysaccharide (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 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 Phellinus Linteus Polysaccharide (PLP) prior to and after ionizing irradiation in mice significantly promoted recovery of hematopoiesis, as indicated by bone marrow and spleen hematopoietic colonies.

Conclusion

Our experimental results show that Phellinus Linteus Polysaccha ride (PLP) can accelerate restoration of radiation induced hemato poietic damage in mice.

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