Effect of panaxatriol on hematogenesis and granulocyte-macrophage colony stimulating factor in radiation injured mice

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ABSTRACT

Objectives: To assess the recovery effect of panaxatriol (PT) on myeloid hemopoiesis in radiation injured mice, and analyze the underlying mechanism.

Methods: This study was carried out in the Animal Center of Shandong University, Jinan, China, during March to September 2006. Forty-five inbred albino mice were separated randomly into 3 groups: control group, radiation group, and radiation + PT group (200 mg/kg/d, 3 weeks). Peripheral blood cells were detected by globuli meter, $CD34^+$ cells in bone marrow were detected by flow cytometry, and the protein expression of granulocyte-macrophage colony stimulating factor (GM-CSF) was detected by immunocytochemistry.

Results: The numbers of peripheral blood cells and bone marrow $CD34^+$ cells, and the expression of GM-CSF in the radiation group were lower than in the control group. After treatment with PT, the numbers of peripheral blood cells and $CD34^+$ cells, and the expression of GM-CSF increased significantly.

Conclusions: Panaxatriol can relieve myelosuppression induced by radiation injury. The abilities of regulating the expression of hemopoietic growth factor GM-CSF and promoting the maturation of bone marrow cells may be responsible for some of these beneficial effects.

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Radiotherapy is an effective treatment for many types of cancer, however, serious side effects such as defects in hemopoiesis (resulting in low numbers of circulating blood cells, and increased susceptibility to infection and hemorrhage)[1,2] may occur during and after treatment, and often lead to the discontinuity of radiotherapy. Protection, therefore, from the undesirable effects of radiotherapy, primarily from myelosuppression, is a crucial problem to be coped with. Granulocyte-macrophage colony stimulating factor (GM-CSF) is a positive hemopoietic growth factor, which has a broad spectrum of activity, and can support the growth and maturation of early precursor cells in bone marrow into mature granulocytes, mononuclear phagocytes, and eosinocytes.[3] Previous studies have demonstrated that GM-CSF protects hematopoietic tissues from radiation-induced death and the host from infection by reducing the radiation damage of primitive hematopoietic cells, and enhancing the function of mature neutrophils.[4,5] Panax ginseng, a traditional Chinese herb, has been extensively used for preventive and therapeutic purposes for thousands of years. Ginseng has a wide range of pharmacological activities including immunomodulatory effect, anti-inflammatory activity, anti-tumor activity, improvement of physical stamina, and stimulation of the appetite, and is also reported to have a radioprotective effect.[6,7] Panaxatriol (PT), one major active component of ginseng, has been shown to reverse radiation-induced damage.[7,8] Some studies have indicated that PT protects the chromosomes of bone marrow cells,[9] immune organs,[10] pancreas,[11] and reproductive endocrine axis,[12] from x-ray radiation. However, to our knowledge, there is no report on the effect of PT on radiation-induced myelosuppression. Thus, we hypothesized that a PT supplement could antagonize the hemopoietic damage induced by radiation injury. The present study was undertaken to confirm the above hypothesis and explore the underlying mechanism.

Methods. ***Animals and study design.*** This study was carried out in the Animal Center of Shandong University, Jinan, China, during March to September 2006, and all experiments were conducted according to the institution's guidelines for the care and use of laboratory animals in research, and approved by the Animal Care Committee of Shandong University. Forty-five inbred albino mice weighing 18-20g were purchased from the Animal Center of Shandong University. They were kept in an animal room at an ambient temperature of 20±1°C under a 12-hour dark-light cycle. Animals were allowed a period of one week acclimatization prior to entry into any experimental protocol, during which they were given free access to sterile normal food and water. After one week of acclimatization, the mice were randomized into 3 groups: control group, 15 healthy mice were fed under the same conditions without any treatment. Radiation group (15 mice), mice were placed in ventilated plexiglas containers and exposed to gamma rays from the ^{60}Co source (provided by Shandong Academy of Agricultural Science). Each animal received a whole dose of 3.5 Gy radiations at the dose rate of 1.27 Gy/minute (min) at ambient temperature. Radiation + PT group (15 mice), the radiation injury procedure was the same, however, PT was given by oral administration at the dose of 200 mg/kg once a day for 3 weeks after irradiation.

Hematological examination. Twenty microliters of blood was collected from each mouse in the 3 groups by tail cutting on the 1st, 7th, 13th, and 18th day after irradiation. The white blood cell count (WBC), red blood cell count (RBC), and platelets (PLT) in peripheral blood were counted, and the contents of hemoglobin (HGB) were determined using a Sysmex F-820 globuli meter (Sysmex Company, Japan).

Preparation of bone marrow cell suspension. On the 21st day after irradiation, mice were killed by decapitation, and the femora were dissected under sterile conditions. The bone marrow cells were obtained, and a cell suspension was prepared in phosphate-buffered saline (PBS) by repeated flushing of the bone marrow through a 6-gauge needle, anticoagulating with natrium citrinum and filtrating through a 4-gauge needle. Part of the cell suspension was smeared for detecting GM-CSF expression by immunocytochemistry and part was fixed in 2% paraformaldehyde at 4°C for analyzing $CD34^+$ cells by flow cytometry.

Assaying the CD34+ cells of bone marrow. The cell suspension was centrifuged at 2500 g/min for 8 minutes and resuspended in 2 ml PBS. Thirty microliters of normal mice serum was used to block nonspecific antibody binding sites. Bone marrow cells were incubated with fluorescein isothiocyanate (FITC) labeled rat anti-$CD34^+$ MoAb (Becton Dickinson Company) for 30 minutes at 4°C, 2 ml of red blood cell lysate was added to react for 4 minutes and then washed twice in PBS, then stained with propidium iodide (PI) and detected by FACScan (Becton Dickinson Company).

Examining the expression of GM-CSF. A single cell suspension was smeared on microscope slides primed by polylysine. After fixation with 4% paraformaldehyde at 4°C for 10 minutes, the cells were incubated in 0.3% hydrogen peroxide in methanol for 20 minutes to block endogenous peroxidase. The cells were blocked with 1:50 goat serum for 30 minutes at room temperature, and then reacted with rabbit anti-GM-CSF antibody appropriately diluted in PBS (1:100) overnight at 4°C (rabbit anti-GM-CSF kit, Boster Biotechnology Corp, Wuhan, China). The cells were incubated for 30 minutes with biotinylated anti-rabbit gamma immuno-globulin G (dilution, 1:100), washed in PBS, and incubated for a further 30 minutes with streptavidin biotin-peroxidase complex ([SABC] staining kit, Boster Biotechnology Corp, Wuhan, China). The stain was then developed in diaminobenzidine solution at room temperature for 5-15 minutes until a satisfactory color reaction was achieved. The slides were mounted and observed by light microscopy (Olympus, Tokyo, Japan). The positive cells expressing GM-CSF were scored by computer-assisted image analyzer (Institute of Biomedical-engineering, Beijing, China).

Statistical analysis was performed with the Statistical Package for Social Sciences, version 11.0 software. The data were presented as mean±standard error of mean. Results were analyzed by one-way analysis of variants. A $p<0.05$ was considered statistically significant.

Results. Analyses from the peripheral blood cells showed that the WBC, RBC, PLT, and HGB contents in radiation injured mice were decreased significantly. The change trend was that approximately 2 weeks after irradiation, the counts of WBC, RBC, and HGB decreased to the lowest level and then recovered gradually and approximately one week after irradiation, the counts of PLT decreased to the lowest level and then recovered gradually, however, this was still lower than the control group. At each time point, the counts of WBC, RBC, PLT, and HGB in the PT group were higher than in the radiation injury group (**Figure 1**). As shown in **Figure 2**, the percentage of bone marrow $CD34^+$ cells, assessed by flow cytometry, was significantly lower in the radiation mice than in the control (1.64±0.45% versus [vs] 28.11±7.51%, $p<0.01$). After treatment with PT, the percentage was increased markedly (12.86±7.21% vs 1.64±0.45%, $p<0.01$). Immunocytochemical SABC staining indicated that on the 21st day after irradiation, the positive bone marrow cells expressing GM-CSF in radiation injured mice decreased by 56.31% compared

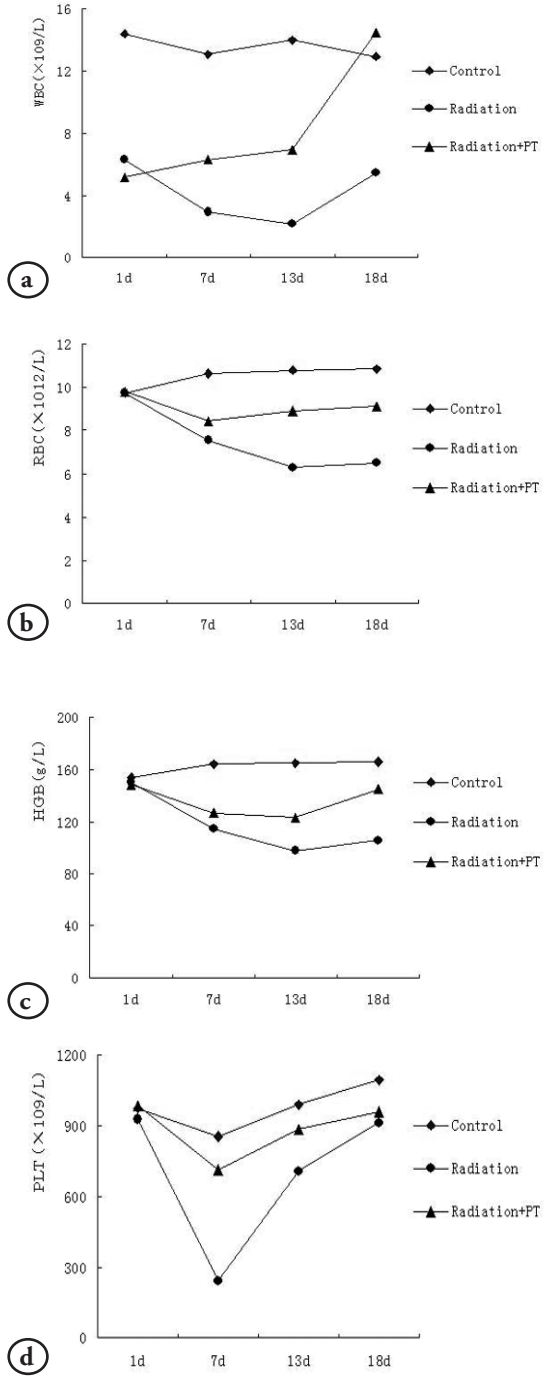

Figure 1 - Effect of panaxatriol (PT) on peripheral blood cells in radiation mice showing the changes of **a**) white blood cell (WBC), **b**) red blood cell (RBC), **c**) hemoglobin (HGB), and **d**) platelets (PLT) in the peripheral blood on the 1st, 7th, 13th, and 18th day after the mice were irradiated and treated with PT. The number of WBC, RBC, HGB, and the PLT decreased and gradually increased in the radiation group mice, at each time point, the numbers of peripheral blood cells were higher in radiation + PT group than in radiation group.

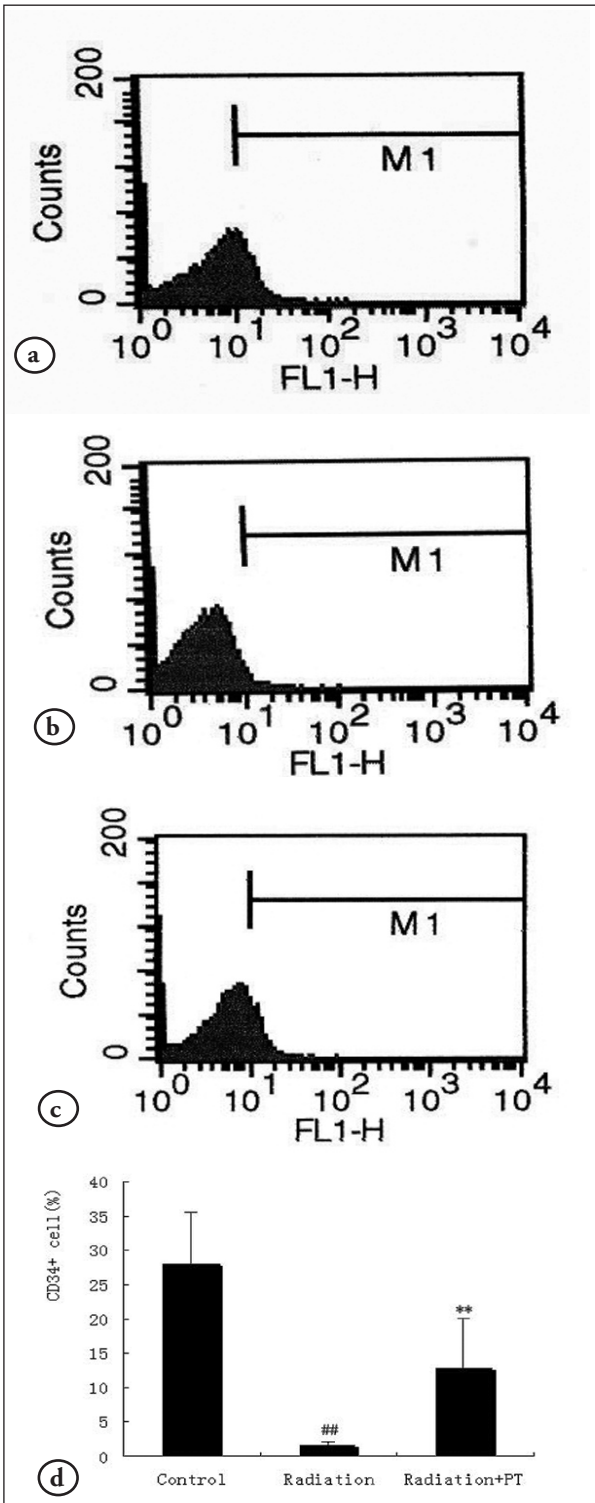

Figure 2 - Effect of panaxatriol (PT) on bone marrow $CD34^+$ cells in radiation mice. Representative histograms of the flow cytometry assay for **a**) control, **b**) radiation, and **c**) radiation + PT group; **d**) percentage of $CD34^+$ cells in each group. Data were presented as mean±standard error of mean. ##p<0.01 significantly different from control group, **p<0.01 significantly different from radiation group.

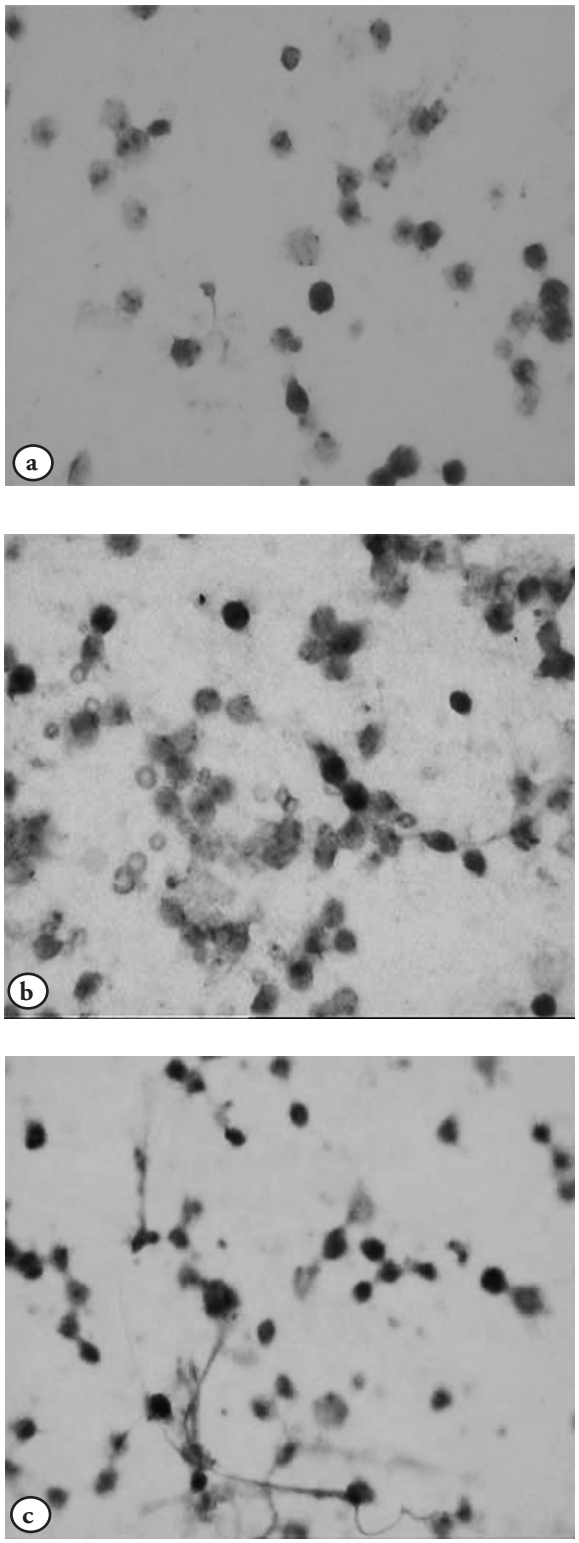

Figure 3 - Effect of panaxatriol (PT) on granulocyte-macrophage colony stimulating factor (GM-CSF) expression in radiation mice, a) control group, b) radiation group, c) radiation + PT group. (GM-CSF-specific antibody binding capacity staining x 400)

with the normal group (19.93±4.06% vs 45.62±6.70%, $p<0.01$). After treatment with PT, the positive rate increased markedly (36.36±3.42% vs 19.93±4.06%, $p<0.01$) (**Figure 3**).

Discussion. The results of our study showed an anti-myelosuppression effect of PT on radiation injured mice, which could increase the number of peripheral blood cells, enhance the percentage of $CD34^+$ cells in bone marrow and promote the expression of GM-CSF. The hemopoietic system is known to be highly sensitive to ionizing radiation and the total body radiation of the animal first leads to hemopoietic damage. It is characterized by the decrease of circulating leukocytes, platelets and erythrocytes, and the death of animals due to infection, hemorrhage, and anemia.[2,13] In line with these reports, this study demonstrated that, after radiation by ^{60}Co gamma-rays, the contents of WBC, RBC, HGB, and PLT in peripheral blood were significantly decreased, whereas supplementation with PT resulted in a remarkable recovery of the peripheral blood cells.

The CD34 antigen is a highly glycosylated transmembrane glycoprotein expressed on the surface of hematopoietic stem and progenitor cells. CD34 expression decreases as hematopoietic stem cells differentiate, and is absent in all mature blood cells.[14] For research and clinical use, CD34 is the most commonly used marker to obtain enriched populations of hematopoietic stem cells and progenitors.[15] In transplantation, $CD34^+$ bone marrow cells can rescue mice from the lethal effects of total-body radiation.[14] In our study, ^{60}Co gamma-rays induced remarkable decrease of $CD34^+$ cells in mice. This result was consistent with some other studies.[14,16] Whereas treatment with PT resulted in a significant increase of CD34+ cells, which indicated that PT could decrease the radiation-induced hemopoietic damage and subsequently promote the restoration of peripheral blood cells.

The production of blood cells is a complex system involving both proliferation and differentiation, and is regulated by a network of cytokines and hemopoietic growth factors. Amongst these, GM-CSF stimulates both proliferation and differentiation of hemopoietic precursor cells and modulates the function of the mature progeny.[17] In clinics, GM-CSF has been used as a hematopoietic growth factor and a host defense regulator to stimulate hematopoietic cell proliferation after chemotherapy and autologous or allogeneic bone marrow transplantation.[18,19] In our study, the expression of GM-CSF in bone cells increased significantly in radiation mice treated with PT compared with untreated animals. It suggested that the effect of PT on myeloid hematogenesis may relate to the upregulation

of GM-CSF expression. However, there were some limitations in the present study. First, the duration of the treatment was relatively short (3 weeks). In this regard, our results were considered to be short-term effects, which may be different from long-term effects. Second, this study was carried out in an experimental model of radiation injury that differed in human. But our results were consistent with some other observations that PT could decrease radiation-induced damage.[7,8] Therefore, it will be of great interest to study whether PT might provide long-term benefits in preventing radiation-induced injury in patients. Finally, the number of the animals was relatively small, thus these conclusions need to be further investigated.

In conclusion, this study indicated that PT could promote the recovery of hematopoietic stem/progenitor cells and peripheral blood cells efficiently and reverse the radiation-induced myelosuppression in mice. The mechanisms were possibly associated with the regulatory effect of PT on GM-CSF expression. It is promising for PT to be used as a protective agent for radiotherapy in clinics.

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