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Protective effects of paeoniflorin and albiflorin on chemotherapy-induced myelosuppression in mice

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[ABSTRACT] *Paeonia lactiflora* root (baishao in Chinese) is a commonly used herb in traditional Chinese medicines (TCM). Two isomers, paeoniflorin (PF) and albiflorin (AF), are isolated from *P. lactiflora.* The present study aimed to investigate the protective effects of PF and AF on myelosuppression induced by chemotherapy in mice and to explore the underlying mechanisms. The mouse myelosuppression model was established by intraperitoneal (i.p.) injection of cyclophosphamide (CP, 200 mg·kg^{-1}). The blood cell counts were performed. The thymus index and spleen index were also determined and bone morrow histological examination was performed. The levels of tumor necrosis factor-α (TNF-α) in serum and colony-stimulating factor (G-CSF) in plasma were measured by Enzyme-Linked Immunosorbent Assays (ELISA) and the serum levels of interleukin-3 (IL-3), granulocyte-macrophagecolony-stimulatingfactor (GM-CSF), and interleukin-6 (IL-6) were measured by radioimmunoassay (RIA). The levels of mRNA expression protein of IL-3, GM-CSF and G-CSF in spleen and bone marrow cells were determined respectively. PF and AF significantly increased the white blood cell (WBC) counts and reversed the atrophy of thymus. They also increased the serum levels of GM-CSF and IL-3 and the plasma level of G-CSF and reduced the level of TNF-α in serum.. PF enhanced the mRNA level of IL-3 and AF enhanced the mRNA levels of GM-CSF and G-CSF in the spleen. PF and AF both increased the protein levels of GM-CSF and G-CSF in bone marrow cells. In conclusion, our results demonstrated that PF and AF promoted the recovery of bone marrow hemopoietic function in the mouse myelosuppression model.

[KEY WORDS] Albiflorin; Paeoniflorin; Chemotherapy; Myelosuppression

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Introduction

The root of *Paeonia lactiflora* Pall (called *baishao* in Chinese), belonging to the Ranunculaceae family, is commonly used in traditional Chinese medicine (TCM). Many active constituents have been identified from this herb, including paeoniflorin (PF), albiflorin (AF), oxypaeoniflorin, paeonilactinone, benzoyloxypaeoniflorin, and lactinolide [1]. It is well documented that PF possesses remarkable effects in the treatment of pain [2-3], muscle spasm [4-5], inflammation [6], and neurodegenerative disorders [7-10]. However, there are few reported pharmacological researches on AF, and the study of its hematopoietic effects is lacking. More recently, we have found that PF and AF could suppress radiation- and chemotherapy-induced myelosuppression [11-13]. In a recent report, Xu *et al.* has identified an active fraction from baishao, a combination of paeoniflorin and albiflorin, which has ameliorative effects on myelosuppression induced by radiotherapy and chemotherapy [14]. Jiang *et al.* have proven the anti-inflammation effect of baishao extract on cyclic nucleotides (cAMP)-phosphodiesterase activity in a rat arthritis model [5].

Cancer is a leading cause of death worldwide. Chemotherapy is widely used to treat cancers, but may cause severe myelosuppression, resulting in a decrease in peripheral blood cell counts, which may further cause infection, anemia, hemorrhage, and severely affect the outcome of chemo-therapy [15]. Growth factors, including granulocyte colony-

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stimulating factor (G-CSF) [16-17] and granulocytemacrophage colony-stimulating factor (GM-CSF) [18] are generally effective and safe for replenishing red blood cells and platelets in the clinic. Considering that hematopoiesis is a diverse and complex process, which is regulated by various hematopoietic cytokines, such as G-CSF, GM-CSF, IL-3, IL-6 and TNF-α, an effective approach to enhancing the recovery from myelosuppression is inducing the activation of hematopoietic cytokines [19]. Transfusions and growth factor injections can accelerate hemopoietic recovery during cancer therapy and indeed improve the bone marrow performance to certain extent [20]. Nowadays, the research community begins to realize the potential therapeutic effects traditional Chinese medicines (TCM) on myelosuppression [21].

Based on our previous studies [11-13], we hypothesized that PF and AF, two characteristic isomers in *P. lactiflora.*, have hematopoietic effect and can be used for prevention and/or treatment of chemotherapy-induced myelosuppression. To test this hypothesis, we examined the effects of PF and AF on cyclophosphamide-induced myelosuppression in mice. We checked the hematopoietic functions of PF and AF, including changes in peripheral leukocyte count, thymus index, spleen index, and bone morrow histology. We also explored the underlying mechanisms for AF and PF in the protection against chemotherapy- induced myelosuppression by measuring the levels of hematopoiesis-related cytokines (including G-CSF, GM-CSF, IL-3, IL-6 and TNF-α) in plasma or serum and their mRNA and protein expression levels in spleens and/or bone marrow cells.

Materials and Methods

Materials and drugs

Paeoniflorin (PF) and Albiflorin (AF) were prepared in our laboratory (Patent No. ZL 201110184287.4, China). The purity of PF (purity = 98.6%) and AF (purity = 96.7%) were measured by reverse-phase high-performance liquid chromatography (HPLC) coupled with ultraviolet detection, according to Chinese Pharmacopoeia [1] (2015, Beijing, China). The representative HPLC chromatograms and chemical structures of the two isomers are shown in Fig. 1. PF and AF were freshly dissolved in sterile normal saline solution.

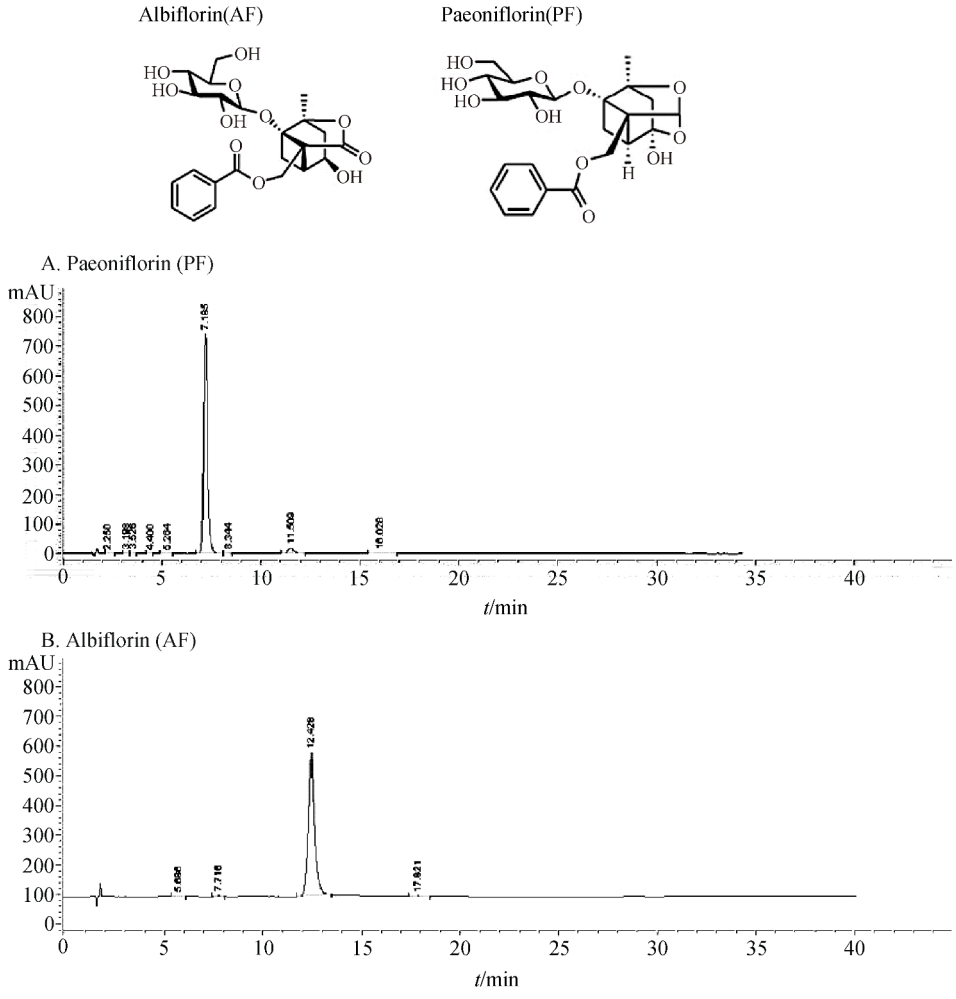

Fig. 1 Chemical structures and HPLC chromatograms of Paeoniflorin (PF) (a) and Albiflorin (AF) (b)