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 medicine (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 marrow 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-macrophage colony-stimulating factor (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 hematopoietic function in the mouse myelosuppression model.

[KEY WORDS] Albiflorin; Paeoniflorin; Chemotherapy; Myelosuppression


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 chemotherapy [15]. Growth factors, including granulocyte colony-
stimulating factor (G-CSF) \cite{16-17} and granulocyte-macrophage colony-stimulating factor (GM-CSF) \cite{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-\(\alpha\), an effective approach to enhancing the recovery from myelosuppression is inducing the activation of hematopoietic cytokines \cite{19}. Transfusions and growth factor injections can accelerate hemopoietic recovery during cancer therapy and indeed improve the bone marrow performance to certain extent \cite{20}. Nowadays, the research community begins to realize the potential therapeutic effects traditional Chinese medicines (TCM) on myelosuppression \cite{21}.

Based on our previous studies \cite{11-13}, we hypothesized that PF and AF, two characteristic isomers in \textit{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 marrow 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-\(\alpha\)) 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 \cite{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.

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**Fig. 1** Chemical structures and HPLC chromatograms of Paeoniflorin (PF) (a) and Albiflorin (AF) (b)
Cyclophosphamide injection was obtained from Jiangsu Shengdi Pharmaceutical Co., Ltd. (Jiangsu, China; batch No. 15111125). The counts of white blood cells (WBC), red blood cells (RBC), and platelets (PLT) and the hemoglobin (HGB) level were determined using a Sysmex XE-2100 complete automatic hemanalysis instrument (Kobe, Japan). Mouse tumor necrosis factor-α (TNF-α) and colony-stimulating factor (G-CSF) Enzyme-Linked Immunosorbent Assay (ELISA) kits and interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-6 (IL-6) radioimmunoassay (RIA) kits were purchased from Beijing Shino-UK Institute of Biological Technology, Inc. (Beijing, China). Super RT cDNA kit and primary antibodies and secondary antibody of G-CSF, GM-CSF, IL-3, IL-6 and TNF-α were also purchased from Beijing Sino-UK Institute of Biological Technology, Inc. Primers used for real-time quantitative PCR (RT-qPCR) were designed and synthesized by Beijing Sino-UK Institute of Biological Technology, Inc. The purity of all chemical reagents used in the present study was of at least analytical grade.

**Animals**

Kunming mice (male, 6 weeks old, weighing 18–22 g) were obtained from Vital River Co., Ltd. (Beijing, China) and maintained at controlled temperature (22 ± 2 °C) and humidity (50% ± 10%) with a 12 h/12 h light/dark cycle. All efforts were made to minimize the pain of animals. The experimental protocol was approved by the Ethics Committee of Beijing University of Chinese Medicine (No. Kj-dw-18-20150821-01).

**Cyclophosphamide (CP) treatment**

After seven days of acclimatization, the mice were divided into six groups (n = 10 per group): (1) normal control group (Control), (2) cyclophosphamide treatment group (Model), (3) low-dose PF treatment group (15 mg·kg⁻¹ PF), (4) high-dose PF treatment group (30 mg·kg⁻¹ PF), (5) low-dose AF treatment group (15 mg·kg⁻¹ PF) and (6) high-dose AF treatment group (30 mg·kg⁻¹ AF).

All the drug treatment groups (Groups 4-6) were intragastrically (i.g.) pretreated with PF (15 and 30 mg·kg⁻¹) or AF (15 and 30 mg·kg⁻¹) for 7 days. The Cyclophosphamide (CP) treatment was given according to Yang’s method [15]; the mice in Groups 2-6 were injected intraperitoneally with a single dose of CP (200 mg·kg⁻¹, 0.2 mL·10 g⁻¹) on Day 8, and the normal control group received an equal volume of saline. Immediately after CP treatment, the mice in groups 3-6 were administrated of PF and AF (i.g., 15 and 30 mg·kg⁻¹, 0.2 mL·10 g⁻¹) for 3 days. The control and CP groups were administered an equivalent volume of saline water, once a day for 3 consecutive days.

**Determination of peripheral leukocyte count, thymus index and spleen index**

On Day 11, blood samples (20 mL) were collected from the eye socket vein and white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB) and platelets (PLT) were counted using a cell counter (Sysmsex XE-2100, Kobe, Japan). After the last drug administration, body weights of mice were measured. Blood samples were collected by extracting eyeballs and placed into test tubes with or without ethylenediaminetetraacetic acid (EDTA) for further analysis. After the mice were sacrificed by cervical dislocation, the thymus gland and spleen were excised and weighed; the thymus index and spleen index were calculated as follows: (organ weight/ body weight) × 1000.

**Bone marrow histological examination**

Femoral bones of three mice in each group were removed, fixed in 4% paraformaldehyde, and then treated with a formic acid-sodium citrate decalcification solution for 5 days. The bones were embedded in paraaffin and sectioned at 5-μm thickness for stained with H&E for the histological examination of bone marrow under a light microscope.

**Determination of the levels of G-CSF, GM-CSF, IL-3, IL-6, and TNF-α in plasma or serum**

Plasma or serum samples were prepared from the blood samples collected from the mice, and the levels of G-CSF, TNF-α, GM-CSF, IL-3 and IL-6 were measured using ELISA or RIA kits.

**Analysis of mRNA expressions of G-CSF, GM-CSF, IL-3, IL-6 and TNF-α in spleen**

The spleens were homogenized with RNA isoplus and centrifuged twice at 4°C and the total RNA was extracted from the supernatant fraction and RNA purity was assessed by spectrophotometric measurements. Then total RNA from each sample was reverse-transcribed into cDNA using a Super RT cDNA kit, and the synthesized cDNA was used for RT-qPCR amplification using SYBR green Realtime PCR Master Mix. The sequences of the primers used for PCR are shown in Table 1. The cycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 60 s, and 75 °C for 20 s. The RT-qPCR analysis was performed with the Light Cycler 480 RT-qPCR System (Roche, Basel, Switzerland). The results of relative mRNA expression in each group were semi quantitated using the comparative Cₚ method, setting normal control as 1.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward (5'–3' )</th>
<th>Reverse (5'–3' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>AGGAGCGGAGACCCCACATACA</td>
<td>AGGGGGCTAAGCAGTTGTT</td>
</tr>
<tr>
<td>IL-3</td>
<td>GCCGTGCTACATCTCGGAA</td>
<td>GGTAGGAAGAGACGGGCAA</td>
</tr>
<tr>
<td>IL-6</td>
<td>GTCCGGGAGGAGACTTCC</td>
<td>CTGCAAAGTGATCATCTGGT</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>TTAAATTCTTCTGGCGATTGG</td>
<td>CAGGAAGCTTACGAGGGTTT</td>
</tr>
<tr>
<td>TNF-α</td>
<td>ACCCTCACACTCAAAACCA</td>
<td>ATAGCAAATCGGGCTAGG</td>
</tr>
<tr>
<td>G-CSF</td>
<td>CGCATGAGACTAATGGGTTA</td>
<td>GACGGGCTCTGAGGCATTT</td>
</tr>
</tbody>
</table>
Western blot analyses for the protein levels of G-CSF, GM-CSF, IL-3, IL-6 and TNF-α in bone marrow

Three mice in each group were sacrificed by cervical dislocation and the femoral bones were separated, then briefly immersed in 75% ethanol, and rinsed three times in phosphate buffer solution (PBS) containing antibiotics (Penicillin–Streptomycin) under sterile conditions to collecting the bone marrow. According to the Ma’s method \[32\]. The total protein in bone marrow cells was extracted with lysis buffer (50 mmol L\(^{-1}\) Tris, pH 8.0, 150 mmol L\(^{-1}\) NaCl, 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, 100 mg mL\(^{-1}\) phenylsulfonyl fluoride, 2 mg mL\(^{-1}\) aprotinin, 1 mg mL\(^{-1}\) pepstatin, and 10 mg mL\(^{-1}\) leupeptin), and 50 mg protein from each sample was resolved on a 10% sodium dodecyl sulfate polyacrylamide gel. The fractionated proteins were transferred to an immobilon polyvinylidene fluoride membrane, which was then probed with primary antibodies against G-CSF, GM-CSF, IL-3, IL-6, and TNF-α (Bioss. Inc, Beijing, China).

Statistical analysis

The results were expressed as means ± SD. Statistical differences were determined by one-way analyses of variance (ANOVA) and Student’s \(t\) tests. \(P < 0.05\) was considered statistically significant.

Results

Effects of PF and AF on peripheral blood cells

As shown in Table 2, the white blood cell counts (WBC) in CP model group were significantly reduced compared to that in the normal control group \((P < 0.001)\). The mice in the 30 mg kg\(^{-1}\) PF and 30 mg kg\(^{-1}\) AF groups showed significantly elevated WBC counts, compared with the model group. \((P < 0.05, P < 0.01)\). Table 2 also indicates that the platelet counts (PLT) in CP model group were significantly decreased, compared to that in the normal control group \((P < 0.001)\). 30 mg kg\(^{-1}\) AF elevated the number of PLT, compared with the model group \((P < 0.05)\).

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC</th>
<th>RBC</th>
<th>HGB</th>
<th>PLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.81 ± 1.48</td>
<td>7.44 ± 0.42</td>
<td>126.9 ± 8.50</td>
<td>996.9 ± 163.68</td>
</tr>
<tr>
<td>Model</td>
<td>2.24 ± 0.64***</td>
<td>7.04 ± 0.57</td>
<td>121.2 ± 6.01</td>
<td>743.9 ± 194.97***</td>
</tr>
<tr>
<td>15 mg kg(^{-1}) PF</td>
<td>2.49 ± 1.21</td>
<td>7.06 ± 0.44</td>
<td>121.3 ± 7.20</td>
<td>766.0 ± 112.51</td>
</tr>
<tr>
<td>30 mg kg(^{-1}) PF</td>
<td>3.70 ± 1.57*</td>
<td>7.32 ± 0.43</td>
<td>127.6 ± 5.80</td>
<td>759.7 ± 130.85</td>
</tr>
<tr>
<td>15 mg kg(^{-1}) AF</td>
<td>2.62 ± 1.21</td>
<td>7.13 ± 0.64</td>
<td>115.7 ± 7.89</td>
<td>839.0 ± 148.58</td>
</tr>
<tr>
<td>30 mg kg(^{-1}) AF</td>
<td>3.99 ± 1.32**</td>
<td>7.20 ± 0.86</td>
<td>122.8 ± 13.77</td>
<td>891.3 ± 120.36**</td>
</tr>
</tbody>
</table>

\(^* P < 0.05, ^{**} P < 0.01, ^{***} P < 0.001\) vs the control group; \(^{+} P < 0.05, ^{++} P < 0.01, ^{+++} P < 0.001\) vs the model group.

Effects of PF and AF on the thymus index and spleen index

As shown in Fig. 2, the mean thymus index in the model mice was significantly decreased, compared with that in the normal control group \((P < 0.001)\). 30 mg kg\(^{-1}\) PF and 30 mg kg\(^{-1}\) AF significantly increased the thymus index, compared with the model control \((P < 0.01\) and \(P < 0.001\), respectively). Fig. 2 also indicates that the spleen index in model group was decreased, compared with the normal control \((P < 0.05)\). However, AF or PF had no effect on the spleen index, compared with the model group.

Effects of PF and AF on bone marrow histopathology

As shown in Fig. 3, the color of the bone marrow tissue of normal mice was uniform, and the architectures of periosteum, cavitas medullaris and cartilage cells were clear (Fig. 3A). Whereas the bone marrow of model group showed the number of nucleated myelocytes (blue) in the bone marrow was reduced and replaced by vacuolation (Fig. 3B). Compared with the model group, 30 mg kg\(^{-1}\) PF group showed a decrease in the vacuole-like degradation and adipose-like cells. 30 mg kg\(^{-1}\) AF also showed an increase in the cell density and decrease in the number of vacuole-like degradation and adipose-like cells (Figs. 3C and 3D, respectively).
Effects of PF and AF on the bone marrow tissue histomorphology of femoral bone (H&E staining, upper panels: 1 × 200, lower panels: 1 × 400) from normal group (A1 and A2), model group (B1 and B2), 30 mg·kg⁻¹ PF treatment groups (C1 and C2) and 30 mg·kg⁻¹ AF treatment groups (D1 and D2)

Effects of PF and AF on the hematopoiesis-related cytokines

As shown in Table 3, compared to the normal control group, the serum level of GM-CSF and the plasma level of G-CSF in the model group were significantly decreased (both \( P < 0.001 \)). Compared with the model group, the levels of GM-CSF in 30 mg·kg⁻¹ PF and 30 mg·kg⁻¹ AF groups were significantly increased, (both \( P < 0.001 \)), and the G-CSF levels in 15 mg·kg⁻¹, 30 mg·kg⁻¹ PF, and 30 mg·kg⁻¹ AF groups were significantly increased ( \( P < 0.05 \), \( P < 0.01 \), and \( P < 0.001 \), respectively).

The CP model group also showed a decrease in IL-3 level in serum, compared with the normal control group ( \( P < 0.001 \)). 30 mg·kg⁻¹ PF and 30 mg·kg⁻¹ AF administration increased the level of IL-3, compared with the model group ( \( P < 0.01 \) and \( P < 0.05 \), respectively). As shown in Table 3, CP induced an increase in IL-6 level, compared with the normal control ( \( P < 0.05 \)). 30 mg·kg⁻¹ AF decreased the level of IL-6, compared with the model group ( \( P < 0.05 \)). In addition, TNF-\( \alpha \) was significantly increased in model group, compared with the normal control ( \( P < 0.01 \)), which was prevented by 30 mg·kg⁻¹ PF and 30 mg·kg⁻¹ AF administration ( \( P < 0.01 \) and \( P < 0.05 \), respectively).

Table 3 Effects of PF and AF on the hematopoiesis-related cytokines (\( n = 10 \), means ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>GM-CSF</th>
<th>G-CSF</th>
<th>IL-3</th>
<th>IL-6</th>
<th>TNF-( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>124.60 ± 7.93</td>
<td>131.28 ± 20.20</td>
<td>14.13 ± 1.76</td>
<td>134.27 ± 15.93</td>
<td>58.71 ± 9.26</td>
</tr>
<tr>
<td>Model</td>
<td>88.24 ± 9.89***</td>
<td>92.58 ± 14.40***</td>
<td>10.14 ± 1.46***</td>
<td>149.81 ± 12.29*</td>
<td>73.98 ± 10.78***</td>
</tr>
<tr>
<td>15 mg·kg⁻¹ PF</td>
<td>95.89 ± 18.83</td>
<td>106.21 ± 7.31</td>
<td>9.94 ± 1.60</td>
<td>149.63 ± 22.06</td>
<td>67.01 ± 11.69</td>
</tr>
<tr>
<td>30 mg·kg⁻¹ PF</td>
<td>108.55 ± 12.23***</td>
<td>114.22 ± 8.33**</td>
<td>12.43 ± 1.92**</td>
<td>136.06 ± 14.78</td>
<td>59.94 ± 8.51***</td>
</tr>
<tr>
<td>15 mg·kg⁻¹ AF</td>
<td>96.50 ± 8.79</td>
<td>10.94 ± 7.52</td>
<td>10.22 ± 1.82</td>
<td>146.65 ± 21.32</td>
<td>65.85 ± 14.13</td>
</tr>
<tr>
<td>30 mg·kg⁻¹ AF</td>
<td>114.07 ± 12.13***</td>
<td>115.09 ± 17.17***</td>
<td>12.09 ± 1.95*</td>
<td>132.31 ± 8.74*</td>
<td>58.45 ± 9.09***</td>
</tr>
</tbody>
</table>

\( * P < 0.05 \), \( ** P < 0.01 \), \( *** P < 0.001 \) vs the Control Group; \( * P < 0.05 \), \( ** P < 0.01 \), \( *** P < 0.001 \) vs the Model Group

Effects of PF and AF on the mRNA levels of G-CSF, GM-CSF, IL-3, IL-6 and TNF-\( \alpha \)

As shown in Fig. 4, compared with the normal group, the mRNA level of GM-CSF in the model group was significantly decreased ( \( P < 0.01 \)). Compared to the model group, the mRNA level of GM-CSF was elevated in 30 mg·kg⁻¹ AF ( \( P < 0.05 \)) treatment group, although 30 mg·kg⁻¹ PF showed no significant effect. Compared with the normal group, the mRNA level of G-CSF was significantly decreased in the model group ( \( P < 0.05 \)). Compared to the model group, the mRNA level of G-CSF was elevated in the 30 mg·kg⁻¹ AF group ( \( P < 0.05 \)). In addition, there were no significant differences in the mRNA levels of IL-3, IL-6, and TNF-\( \alpha \) between the normal and model groups. 30 mg·kg⁻¹ PF significantly increased the mRNA level of IL-3, compared with the model group ( \( P < 0.05 \)).

Effects of PF and AF on the protein levels of GM-CSF, G-CSF, IL-3, IL-6 and TNF-\( \alpha \)

As shown in Fig. 5, CP induced decreases in the protein levels of GM-CSF and G-CSF, compared with the control
Fig. 4  Effects of AF on the mRNA levels of GM-CSF, G-CSF, IL-3, IL-6 and TNF-α. The data are expressed as means ± SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 vs the control group; †P < 0.05, ‡P < 0.01, ††P < 0.001 vs the model group.

Fig. 5  Effects of PF and AF on the protein levels of GM-CSF, G-CSF, IL-3, IL-6, and TNF-α. The data are expressed as means ± SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 vs the control group; *P < 0.05, **P < 0.01, ***P < 0.001 vs the model group.

Discussion

Chemotherapy can cause bone marrow suppression, known as myelosuppression [22]. Clinical hematopoietic parameters are routinely used to monitor the adverse effects [21]. In particular, neutropenia (a decrease in the number of white blood cells known as neutrophils) can increase the risk of infection in cancer patients [14]. In the present study, CP was used to induce myelosuppression. Previous studies have demonstrated that, after CP treatment, peripheral blood WBC counts decline to the lowest point on the 3rd day. RBC counts are not changed evidently, which may be owing to their longer lifespan than WBC in the circulation [15, 24]. In the present study, after CP treatment peripheral blood WBC numbers were declined from Days 9 to 11 (data not shown). PF and AF could significantly reverse the decrease in WBC counts on the 11th days, indicating its anti-myelosuppression effects. Meanwhile, the pathological analyses showed that PF and AF improved the histopathology of bone marrow tissues.

Recently, traditional Chinese medicines (TCM) have already attracted great attentions in alleviating the myelosuppression induced by radiotherapy and chemotherapy. For example, Fu fang E’jiao Jiang (FEJ) [25] is a famous TCM formula, which is made of Colla corii Asini, Radix Ginseng Rubra, Fructus Crataegi, and Radix Rehmanniae Preparata and has been approved by China Food and Drug Administration (CFDA) for clinical use to increase the WBC counts in cancer patients undergoing chemotherapy.

Moreover, considering the close relationship between immunity and hematopoiesis [26], the thymus index and spleen index, reflecting the immune function of an organism, were also investigated in the present study. Our results showed that PF and AF increased the thymus indices, implying that PF and AF possess the capacity of alleviating the CP-induced atrophy of hematopoietic organs (also known as immune organs), besides promoting bone marrow hematopoietic function. However, the results also showed that CP caused marked atrophy of the spleen and PF and AF failed to display any detectable effect in this experiment.

To further determine the mechanism underlying the hematopoiesis activity of PF and AF, we analyzed the hematopoiesis-related cytokines in serum or plasma as well as group (both P < 0.05). 30 mg·kg⁻¹ PF and 30 mg·kg⁻¹ AF could increase the levels of GM-CSF and G-CSF, compared to the model group (both P < 0.05). However, there was no significant difference in the protein level of IL-3 between the normal and model groups. As shown in Fig. 5, CP induced increases in the protein levels of IL-6 and TNF-α, compared with the normal control, while the protein levels of IL-6 and TNF-α were elevated in 30 mg·kg⁻¹ PF and 30 mg·kg⁻¹ AF groups compared with the model group, but did not reach the statistical significance level. Interestingly, the IL-6 and TNF-α protein levels were opposite to those in serum, respectively.
their mRNA expressions in spleen and protein levels in bone marrow cells. Both PF and AF improved the G-CSF level in plasma and its mRNA and protein levels, suggesting that they could recover early hematopoietic cells. Both PF and AF increased the levels of GM-CSF and IL-3 in serum, while only PF enhanced the IL-3 mRNA levels and AF enhanced the GM-CSF mRNA expression, indicating that AF and PF regulated the GM-CSF and IL-3 respectively to stimulate proliferation and maturation of granulocyte and macrophage myeloid cells [27], and accelerate the recovery of circulating hematopoietic lineage and stimulates the growth and effector functions of lymphocytes and macrophages [28].

TNF-α is a potent inhibitor, which in turn affects the differentiation of early bone marrow progenitor cells by altering their response to CSFs [29] and accelerate the release of granulocytes from bone marrow [30]. Interestingly, the present study revealed that both PF and AF could not enhance the mRNA and protein levels, while the protein level of TNF-α were opposite to those in serum; the reason for this observation needs to be investigated further. IL-6 is produced by a number of normal and transformed cell lines [31], which can either promote or inhibit the growth of tumor cells. Importantly, IL-6 acts in concert with IL-3 to induce multi-lineage progenitors from murine spleens [32]. In the present study, no significant activation of IL-6 was observed. The effect of PF and AF on other hematopoiesis-related cytokines remains to be explored further in future study.

In conclusion, the results of the present study indicated that PF and AF have protective function against CP-induced myelosuppression and affect immune regulation and hematopoiesis recovery via improvements of various parameters such as WBC, thymus index, and hematopoiesis-related cytokines. Future pharmacological studies should be conducted to uncover other mechanisms underlying the therapeutic effects of PF and AF.

Acknowledgement

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