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Full Length Research Paper

Effects of *Angelica* polysaccharide on erythrocyte immunity and marrow hematopoiesis in chickens

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To investigate the effects of *Angelica* polysaccharide on erythrocyte immunity and marrow hematopoiesis in chickens, *Angelica* polysaccharide was used for the study of immunity and hematinic mechanism in order to provide basis for clinical reference. In this experiment, three gradient dosages (50, 100, 150 mg/kg body weight) of *Angelica* polysaccharide were drenched to the control group and the anemia groups, respectively. The anemia chickling model was made by abdominal injection of cyclophosphamide (CY) for 6 days (80 mg/kg·day). Red blood cell-C3b receptor (RBC-CR1) and red blood cell-immune complex (RBC-IC) rosette rates were measured and analyzed. Ectogenetic semi-solid culture medium of bone marrow hemopoietic progenitor cells was used to observe *Angelica* polysaccharide and separated serum from *Angelica* polysaccharide treatment on the proliferation of colony-forming unit-erythrocyte (CFU-E), burst-forming unit-erythrocyte (BFU-E) and colony-forming unit-granulocyte macrophage (CFU-GM). The results showed that *Angelica* polysaccharide can significantly increase RBC-CR1 rosette rate in the healthy chicken groups ($p<0.01$), but had no more effect on RBC-IC rosette rate ($p>0.05$). At the same time, *Angelica* polysaccharide can restore the RBC-CR1 rosette rate and the RBC-IC rosette rate caused by cyclophosphamide to the normal level. Serum containing *Angelica* polysaccharide can significantly facilitate the proliferation on CFU-E ($p<0.01$), BFU-E ($p<0.01$) and CFU-GM ($p<0.01$), but *Angelica* polysaccharide had no more direct proliferation on CFU-E, BFU-E and CFU-GM. This indicated that *Angelica* polysaccharide had the proliferation on hemopoietic progenitor cells of marrow by the change of hemopoietic factor.

Key words: Polysaccharide, chicken, erythrocyte, immunity, hematopoiesis.

INTRODUCTION

Dozens of Chinese herbal medicinal formulas have been used for promotion of blood production for centuries. Beneficiary effects of medicinal plants have been reported on a series of biological functions such as antioxidants or diuretic (Zhang et al., 2012; Khan et al., 2012a, b). The root of *Angelica Sinensis*, known as Danggui in China, is one of the most popular Chinese herbal medicines and widely used in traditional Chinese medicinal therapy for various diseases as well as a healthful food tonic and spice for thousands of years (Huang and Wei, 2002). Being called the “female ginseng”, it is excellent as an all purpose women's herb (Hardy, 2000). Danggui can be used for anemia due to chronic renal failure (CRF) (Bradley et al., 1999) and can enhance hematopoiesis by stimulating macrophages, fibroblast, lymphocytes in hematopoietic inductive microenvironment and muscle tissue to secrete hematopoietic growth factors (Mak et al., 2006). *A. Sinensis* is contained by more than 80 composite formulae. Modern researches indicate that phthalides, organic acids and their esters, polysaccharides are main chemical components related to the bioactivities and pharmacological properties of Danggui (Yi et al., 2009). *Angelica* polysaccharide (APS), as the main component of Herbal medicine *Angelica*, has the efficacy of enriching

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blood, purifying blood quality, emmenagoue, acesodyne, lenitive and improving circulation (Varga et al., 2010; Wang et al., 2006). It is used frequently in clinical practice and also frequently appears as the main ingredient in prescriptions for bone injuries (Yang et al., 2002). It also has significant functions on immune and hematopoietic system, and better curative effects on anti-inflammation. Recent pharmacological studies demonstrated that APS had radio-protective effects in irradiated mice through modulation of proliferating response of hematopoietic stem cells (Ye et al., 2001). Gastrointestinal protective effects (Cho et al., 2000; Ye et al., 2003) and the mechanism (Ye et al., 2001) of *Angelica* polysaccharide in rats have been reported and APS was known to be protective against ethanol- or indomethacin-induced mucosal damage (Choy et al., 1994). It was also reported that *A. sinensis* crude extract increased the proliferation of gastric epithelial cells through modulation of several proliferation-related genes, including epidermal growth factor (EGF) receptor and ornithine decarboxylase (ODC) and c-Myc (Ye et al., 2003, 2001). Effects of *Angelica* polysaccharide on blood coagulation and platelet aggregation (Yang et al., 2002) and the protective effect of the polysaccharides-enriched fraction from *A. sinensis* on hepatic injury (Ye et al., 2001) were also studied. In cancer cells, *Angelica* polysaccharide has been reported to possess anti-tumor effects (Shang et al., 2003; Tsai et al., 2005) and also exhibited immunostimulating activities both *in vitro* and *in vivo* (Cho et al., 2000). Cyclophosphamide (CY) is a cytostatic agent that produces systemic toxicity especially on cells with high proliferative capacity, while polysaccharides from *Angelica* polysaccharide have been shown to increase the turnover of hemopoietic stem cells (Hui et al., 2006). In the modern society, *Angelica* polysaccharide has a better potential for drug development (Sarker and Nahar, 2004). However, the protective effect of *Angelica* polysaccharide on CY-induced cytotoxicities in both erythrocyte immunity and the hemopoietic was undefined. Any of these actions would extend the therapeutic application of CY in cancer patients in which the herb could be used together with the cytotoxic agents in cancer therapeutic regimen. In this study, we investigated whether *Angelica* polysaccharide could protect the erythrocyte immunity and hematopoiesis from the cytotoxicity of CY in chickens. We also tested the changes of the hemopoietic factors in response to the damage by CY and protection by *Angelica* polysaccharide.

MATERIALS AND METHODS

Treatment of animals

One-day-old Hyline Brown chickens (male) were purchased from Hebei Laboratory Animal Center, housed in cages and lighted for 24 h at the beginning of pretrial period. The chicklings were given free access to feedstuff and water. All the experimental animals were treated in accordance with the guidelines of the Chinese Council for Animal Care.

Preparation of reagents

Angelica polysaccharide and heparin sodium were purchased from Beijing Biochem Co., Ltd. (China). Cyclophosphamide, microzyme, trypan blue, and methylcellulose were purchased from Sigma Biotech Co., Ltd. (USA). FBS, Ficoll, and RPMI-1640 were purchased from Invitrogen Biotech Co., Ltd. (USA). L-glutamine, 2-mercaptoethanol were obtained from Amresco Biotech Co., Ltd. (USA).

Preparation of anemia chicken model (Ji et al., 2004; Mao et al., 2003)

The anemia chicken model was made by abdominal injection daily of cyclophosphamide for 6 days (80 mg/kg·day) starting from 14-day-old. Red blood cells (RBC) and hemoglobin were measured.

Experiment 1. Effects of *Angelica* polysaccharide on erythrocyte immunity in Chickens

Experiment design

Forty 20-day-old normal chickens were randomly divided into four groups with the same number and similar body weight. The healthy chickens in Group I were not given *Angelica* polysaccharide as control, but the healthy chickens in Groups II, III and IV were given the gradient dosages (50, 100 and 150 mg/kg) of *Angelica* polysaccharide respectively. Forty 20-day-old anemia chickens were also randomly divided into four groups with the same number and similar body weight. The anemia chickens in Group V were not given *Angelica* polysaccharide, but the anemia chickens in Groups VI, VII and VIII were given the gradient dosages (50, 100 and 150 mg/kg) of *Angelica* polysaccharide respectively. After 7 days, the blood samples were collected from heart for RBC-CR1 and RBC-IC rosette rates tests.

RBC-CR1 rosette rate examination

The steps were followed with reference to those of previous study (Guo, 2004). 50 μl of diluted erythrocyte was taken and added to 50 μl blood plasma and 50 μl microzyme Figures 1 and 2. The mixture was incubated at 37°C water bath for 30 min, and then 0.25% glutaradehyde (50 μl) was added to the mixture, mixed by gently shaking, and was allowed to stand still for 5 to 10 min. 50 μl mixture was dropped on glass slide, fixed by methanol for one minute, dried naturally, and dyed by Giemsa-dye for 10 to 15 min. The glass slide was washed and observed after air-drying. According to the nine-point principle, nine points from the left, middle, right and up, middle, down were chosen to be observed under the optical microscope, and the erythrocytes showed the red color and the microzymes showed blue. One microzyme conglutinated with two or more red blood cells and was called one rosette. It was observed twice and the average was recorded. Later, 200 erythrocytes were considered and the rosette rate was calculated.

RBC-IC rosette rate examination

The steps were followed by measurement of RBC-IC rosette rate except that blood plasma was instead by normal saline (Guo et al.,

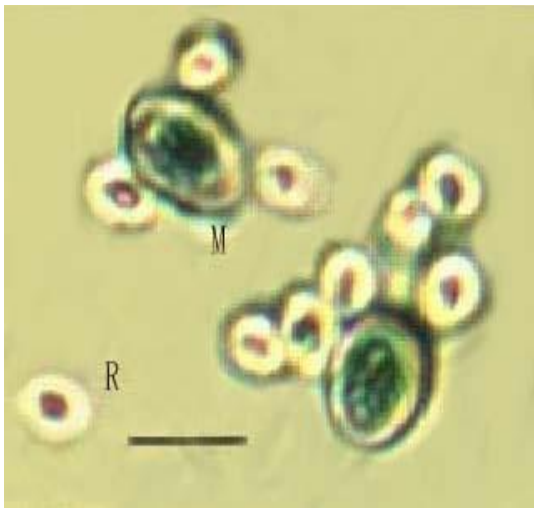

Figure 1. RBC-CR1 rosette. M, Microzyme; R, red blood cell; Scale bar: 50 μm.

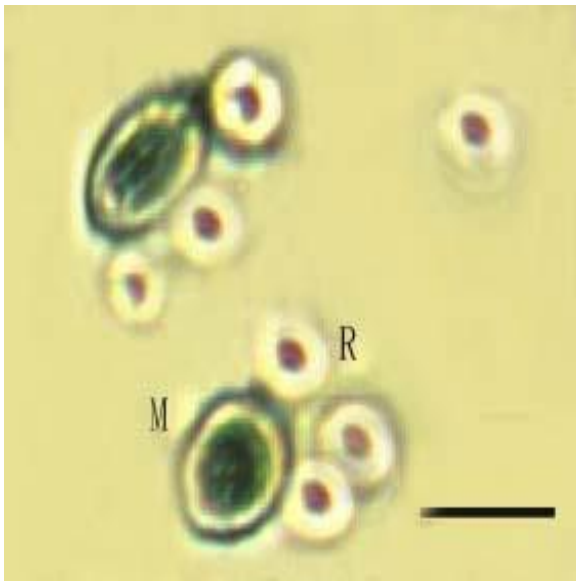

Figure 2. RBC-IC rosette. M, Microzyme, R, red blood cell. Scale bar: 50 μm.

2002, 2003).

Experiment 2. Effects of *Angelica* polysaccharide on proliferation of chicken bone marrow hemopoietic progenitor cells

Effects of serum containing Angelica polysaccharide on proliferation of chicken bone marrow hemopoietic progenitor cells

Preparation of bone marrow cell suspension: Twenty 14-old-day normal chickens with the same body weight were sacrificed, and the bilateral femurs were separated under aseptic conditions. The medullar cavity with RPMI 1640 medium (containing 10 U/ml heparin) were irrigated repeatedly. The cells were collected and lymphocyte separation medium of the same dose was added. After 20-min-centrifugation (2000 r/min), the middle layer was collected and washed by RPMI 1640 medium, and centrifuged three times at 1000 r/min for 10 min each. The cell pellet was suspended in RPMI 1640 medium. The cell viability was checked by Trypan blue. When the cell viability was over 95%, cell concentration was adjusted to 2×10^5 cells /ml (Zheng and Wang, 2002; Yang et al., 2006).

Preparation of culture medium: Methylcellulose semi-solid culture medium was used to culture the colony of hemopoietic progenitor cells and detect the colony proliferation. Culture media were inoculated in 96-well plate at 37°C under 5% CO_2 (Liu et al., 2010). The culture media of colony-forming unit-erythrocyte (CFU-E), burst-forming unit-erythrocyte (BFU-E) and colony-forming unit-granulocyte macrophage (CFU-GM) are as shown in Tables 1 and 2.

Preparation of serum containing *Angelica* polysaccharide: Twenty 7-old-day normal chickens with the same body weight were drenched with *Angelica* polysaccharide (100 mg/kg) for 7 days. The blood was collected from heart, and the serum was separated, filtrated and stored at 4°C.

Effects of serum containing Angelica polysaccharide on the proliferation of chicken bone marrow hemopoietic progenitor cells

The culture media of CFU-E, BFU-E, CFU-GM and bone marrow cells were inoculated in 96-well plates. 25 wells were divided into 5 groups, with 5 replications per group. 0.2 ml distilled water was added in Group I as control. 0.2 ml chicken serum was added in Group II. The gradient dosages (0.1, 0.2 and 0.3 ml) of serum containing *Angelica* polysaccharide were added in Groups III, IV and V, respectively. When CFU-E was cultured for 3 days, colony which contained 8 to 50 cells was one CFU-E under inverted microscope. When BFU-E was cultured for 7 days, colony which contained more than 50 cells was one CFU-E under inverted microscope. When CFU-GM was cultured for 6 days, colony which contained more than 20 cells was one CFU-E under inverted microscope. CFU-E, BFU-E and CFU-GM were all stained with Giemsa staining; the cell morphology was observed and the number of colony was counted (Yang et al., 2007).

Effect of Angelica polysaccharide on proliferation of chicken bone marrow hemopoietic progenitor cells

20 wells were divided into 4 groups, with 5 replications per group. The steps followed were as aforementioned for the effects of serum containing *Angelica* polysaccharide on the proliferation of chicken bone marrow hemopoietic progenitor cells except that serum containing *Angelica* polysaccharide was replaced by *Angelica* polysaccharide. 0.2 ml distilled water was added in Group I as control. The gradient dosages of *Angelica* polysaccharide were added in Groups III, IV and V, respectively at the density of 50, 100 and 150 μg/ml.

Data statistics

The data were taken as mean and standard deviation, using the SPSS11.0 software. The differences were assessed by one way ANOVA.

Table 1. Ingredients of CFU-EBFU-E culture medium.

Ingredient	Amount (ml)	Final concentration (%)
BMC	0.2	10
NCS	0.5	25
10^{-5}M 2- ME	0.2	10
3% L-Glu	0.02	1
EPO	0.3	15
2.2%MC	0.5	25
RPMI-1640	0.3	15

BMC, Bone marrow cell; NCS, new-born calf serum; 2-ME, 2-mercaptoethanol; L-Glu, L-glutamine; EPO, erythropoietin; MC, methylcellulose.

Table 2. Ingredients of CFU-GM culture medium.

Ingredient	Amount (ml)	Final concentration (%)
BMC	0.2	10
NCS	0.5	25
3% L-Glu	0.02	1
GM-CSF	0.3	15
2.2% MC	0.5	25
RPMI-1640	0.5	25

Table 3. Effects of *Angelica* polysaccharide on erythrocyte immunity in healthy chicken.

Group	N	Dosage (mg/kg)	RBC-CR1 rosette rate	RBC-IC rosette rate
I (control of healthy chicken	10	0	7.587 ± 0.508^{aA}	5.967±0.508
II(Low dosage	10	50	7.862 ± 0.299^{aA}	5.797±0.498
III (Middle dosage	10	100	9.591 ± 0.782^{b}	5.994±0.562
IV (High dosage	10	150	12.545 ± 0.597^{cB}	6.453±0.635

The different lowercase and capital letters showed significantly difference at 0.05 and 0.01 level, respectively.

RESULTS

Establishment of anemia chicken model

The anemia model was made by abdominal injection of cyclophphosphamide for 6 days (80 mg/kg•day) when the chickens were 14-day-old. 6 days later, the chickens behaved as follows, sluggish activity, shrinking into oneself, broken-winded, loose-feather, waxy eyelid and nonnasality. Their hemoglobin was just 60% of the normal chicken. These indicated the ideal anemia model was successfully set up.

Effects of Angelica polysaccharide on erythrocyte immunity in healthy chickens

RBC-CR1 rosette rate in Group II (50 mg/kg) had no more change than the control group. RBC-CR1 rosette rate in Group III (100 mg/kg) was notably higher than the control group ($p<0.05$). RBC-CR1 rosette rate in Group IV (150 mg/kg) was significantly higher than the control group ($p<0.01$). However, the RBC-IC rosette rates of the three different dosage groups had no more change than that of the control group (Table 3).

Effects of *Angelica* polysaccharide on erythrocyte immunity in anemia chickens

RBC-CR1 rosette rate in Group V was significantly lower than Group I ($p<0.01$). However, RBC-IC rosette rate in Group V was significantly higher than Group I ($p<0.01$). After the anemia chickens were given Angelia polysaccharide, RBC-CR1 rosette rate gradually increased and restored to the normal level as Group I, at

Table 4. Effects of *Angelica* polysaccharide on erythrocyte immunity in anemia chicken.

Group	N	Dosage (mg/kg)	RBC-CR1 rosette rate	RBC-IC rosette rate
I (control of healthy chicken	10	0	7.587 ± 0.508^{acA}	5.967 ± 0.508^{aA}
V (CY without *Angelica* polysaccharide)	9	0	5.429 ± 0.369^{bB}	8.286 ± 0.541^{bB}
VI (CY with low dosage)	10	50	6.429 ± 0.881^{ab}	6.714 ± 0.656^{a}
VII(CY with middle dosage)	9	100	7.429 ± 0.662^{ac}	6.000 ± 0.588^{aA}
VIII (CY with high dosage)	10	150	7.286 ± 0.565^{ac}	6.000 ± 0.496^{aA}

CY, Cyclophosphamide. The different lowercase and capital letters showed significantly difference at 0.05 and 0.01 level, respectively.

Table 5. Productive rates of CFU-E,BFU-E and CFU-GM.

Group	N	Dosage (ml)	CFU-E	BFU-E	CFU-GM
I (control)	5	0.2	106.000 ± 12.107^{aA}	14.200 ± 5.478^{aA}	24.400 ± 7.570^{aA}
II (serum of healthy chicken)	5	0.2	125.400 ± 10.658^{b}	22.400 ± 5.080^{b}	36.400 ± 5.369^{b}
III (serum containing *Angelica* polysaccharide with low dosage)	5	0.1	137.600 ± 17.441^{cB}	26.200 ± 6.305^{cB}	65.200 ± 9.208^{cB}
IV (serum containing *Angelica* polysaccharide with middle dosage)	5	0.2	138.000 ± 9.425^{cB}	28.400 ± 4.582^{cB}	57.600 ± 9.882^{cB}
Ⅴ (serum containing *Angelica* polysaccharide with high dosage)	5	0.3	120.600 ± 21.255^{ab}	17.200 ± 5.245^{a}	38.400 ± 5.879^{b}

The different lowercase and capital letters showed significantly difference at 0.05 and 0.01 level, respectively.

the same time RBC-IC rosette rate gradually decreased and restored to the normal level as Group I. There were no notable changes in the three different dosage groups compared with Group I ($p>0.05$). RBC-CR1 rosette rate in Group VII (middle dosage) and Group VIII (high dosage) were notably higher than Group V ($p<0.05$). RBC-IC rosette rate in Group VI was notably lower than Group V ($p<0.05$). RBC-IC rosette rate in Group VII and VIII were significantly lower than group V ($p<0.01$). In a word, *Angelica* polysaccharide can restore the decrease of RBC-CR1 rosette rate and the increase of RBC-IC rosette rate caused by cyclophosphamide to the normal level (Table 4).

Effects of serum containing Angelica polysaccharide on proliferation of chicken bone marrow hemopoietic progenitor cells

It was shown in Table 5 that serum containing *Angelica* polysaccharide in Group III (low dosage) and IV (middle dosage) had significant effects on CFU-E than Group I ($p<0.01$). Serum of healthy chicken in Group II had notable effects on CFU-E than Group I ($p<0.05$). Serum containing *Angelica* polysaccharide in Groups III (low dosage) and IV (middle dosage) had notable effects on CFU-E than Group II ($p<0.05$). The serum containing *Angelica* polysaccharide in Groups III (low dosage) and IV (middle dosage) had significant effects on BFU-E than Group I ($p<0.01$). Serum of healthy chicken in Group II had notable effect on BFU-E than Group I ($p<0.05$). Serum containing *Angelica* polysaccharide in Groups III (low dosage) and IV (middle dosage) had notable effects on BFU-E than Group II ($p<0.05$).

The serum containing *Angelica* polysaccharide in Groups III (low dosage) and IV (middle dosage) had significant effects on CFU-GM than Group I ($p<0.01$). Serum of healthy chicken in Group II and serum containing *Angelica* polysaccharide in Group V (high dosage) had notable effects on CFU-GM than Group I ($p<0.05$). Serum containing *Angelica* polysaccharide in Groups III (low dosage) and IV (middle dosage) had notable effects on CFU-GM than Group II ($p<0.05$).

Effect of *Angelica* polysaccharide on proliferation of chicken bone marrow hemopoietic progenitor cells

As shown in Table 6, the different dosages of *Angelica* polysaccharide had no notable effects on CFU-E, BFU-E and CFU-GM than the control group I ($p>0.05$).

DISCUSSION

Many methods could set up the bone marrow inhibition

Table 6. Productive rates of CFU-E, BFU-E and CFU-GM.

Group	N	Dosage (μg/ml)	CFU-E	BFU-E	CFU-GM
I (control)	5	0	106.000±14.107	15.200±5.818	24.400±6.570
II (*Angelica* polysaccharide with low dosage)	5	50	98.400±9.990	15.400±3.226	25.600±5.683
III (*Angelica* polysaccharide with middle dosage)	5	100	104.600±10.565	17.000±3.420	30.800±8.658
IV (*Angelica* polysaccharide with high dosage)	5	150	102.200±8.463	16.400±3.567	26.800±6.125

model, such as radiation damage method, chemical method and immune inducing method. Cyclophphosphamide is the non-cytostatic drug that acts non-specifically on both tumor cells and normal healthy cells with high proliferating capacity like immune cells and GI tissues (Jin et al., 2001; Mao et al., 2002). Cyclophphosphamide exerts its cytotoxicity by cross-linking DNA strands and activation of p53-dependent growth arrest and apoptosis (Ye et al., 2001). It was therefore not surprising that cyclophphosphamide administration resulted in a decrease in the proliferating cell number. The advantage of anemia model made by abdominal injection of cyclophphosphamide is more suitable for the clinical manifestations. Cyclophphosphamide produced myelosuppression manifested as leucopenia and also significantly reduced the blood supply and proliferating cell number. The clinical manifestations of bone marrow inhibition were as follows; whole blood decline, decrease of bone marrow nucleated cells and megakaryocyte. Radioactive ray had direct damage to stem cells and bone marrow microenvironment. Radioactive ray brought down the bone marrow and affected CFU-E, BFU-E and CFU-GM at certain dosage. As an anti-tumor drug, cyclophphosphamide destroyed DNA directly and prevented its replication. The effects of Cyclophphosphamide were marrow inhibition and cell decline, especially granulocyte. Cyclophphosphamide could also damage the bone marrow microenvironment and the proliferation and differentiation of hematopoietic cell, in order to inhibit the hematopoietic function (Ji et al., 2004).

The results indicated that RBC-IC rosette rate of the healthy chickens given *Angelica* polysaccharide was elevated to vary extents compared with the control group. At the same time, RBC-IC rosette rate of healthy chickens given *Angelica* polysaccharide was higher than healthy chickens not given *Angelica* polysaccharide. *Angelica* polysaccharide could cause secondary erythrocyte immunity to be strengthened and affect the coordination of immune function directly or indirectly. The level of RBC-CR1 rosette rate is related to the number and activity of CR1 in the erythrocyte surface.

The main immune function of erythrocyte is removing the immune complex in blood circulation. The level of RBC-IC rate reflects the scavenging speed of RBC-IC. RBC-IC rosette rates of anemia chicken given *Angelica* polysaccharide were lower than anemia chicken not given *Angelica* polysaccharide, which showed *Angelica* polysaccharide improved the removing ability of erythrocyte to immune complex in blood circulation. *Angelica* polysaccharide may activate the activity of CR1 in cytomembrane, decrease the number of C3b receptors, accelerate the clear of immune complex, decrease RBC-IC, and then enhance the immune function of erythrocyte (Yang et al., 2006, 2005; Cui and Chen, 2002).

In this experiment, the results indicated that cyclophosphamide could significantly decrease RBC-CR1 rosette rate and increase RBC-IC rosette rate; so cyclophosphamide could make chicken blood deficiency. *Angelica* polysaccharide could significantly increase RBC-CR1 rosette rate, but have no notable effects on RBC-IC rosette rate of healthy chicken. At the same time, *Angelica* polysaccharide could restore the drop of RBC-CR1 rosette rate and the increase of RBC-IC rosette rate caused by cyclophosphamide to the normal level. The results showed that *Angelica* polysaccharide could not only strength the erythrocyte immunity but also resist the immune restrain caused by cyclophosphamide. *Angelica* polysaccharide had no notable effects on RBC-IC rosette rate that showed there was less immune complex in the circulation of the healthy chicken than that of the anemia chicken.

The results also showed that low dosage (50 mg/kg) of *Angelica* polysaccharide had no notable effects on improving the reduction of erythrocyte immunity, but middle (100 mg/kg) and high dosages (150 mg/kg) could significantly reverse the reduction of erythrocyte immunity in chicken caused by cyclophosphamide. Considering the economical benefit, middle dosage was used to treat chicken anemia. Erythrocyte immunity in chicken could be enhanced after chickens were decocted *Angelica* polysaccharide. Although erythrocyte immunity was the non-specific immunity, it had the promoting and adjusting functions to specific immunity. So we can come to the conclusion that the state of erythrocyte immunity actually reflects the state of organism immunity. In this experiment, *Angelica* polysaccharide had notable effects on erythrocyte immunity. Furthermore, *Angelica* polysaccharide could promote the immune function of organism; consequently gain the purpose of prevention

and treatment.

The study firstly revealed that *Angelica* polysaccharide could promote the hematopoiesis and its regulation of hematopoietic function was in multi-similarity and different ways. On the one hand *Angelica* polysaccharide enriches the blood directly, increases the number of RBC and hemoglobin; on the other hand it regulates the hematopoietic factors, enriches the blood indirectly. Although *Angelica* polysaccharide can stimulate the proliferation of CFU-E, BFU-E and CFU-GM, its action segment and pathway should be further studied. This experiment provided a better basis for the clinical use of hematopoietic (Gui and Yuan, 2000).

Hematopoietic process of organism is an active cell proliferation and differentiation and release process. Pluripotential stem cells are being self-renewed in order to maintain constant number. Pluripotential stem cells change to committed hematopoietic progenitor cell, after further proliferation and differentiation, it was then release to peripheral blood circulation. Other results suggested that *Angelica* polysaccharide could increase reticulocyte of healthy mice, and obviously promote the recovery of erythrocyte, hemoglobin, leukocyte and karyote caused by phenyl hydrazine and ^{60}Co-γ. No matter if healthy or anemia mice, after injecting *Angelica* polysaccharide it obviously stimulates the proliferation and differentiation of hematopoietic progenitor, such as BFU-E, CFU-E, CFU-GM and CFU-Meg. The productivity of CFU-E, BFU-E and CFU-GM were noticeably improved after *Angelica* polysaccharide injection was added to the normal person bone marrow medium. The best concentration is 50 μg/ml. The productivity of CFU-E, BFU-E and CFU-GM were improved by 36.2, 63.6 and 33.4%, respectively,after *Angelica* polysaccharide injection was added to aplastic anemia patient's medium at 50 μg/ml.

Results demonstrated that *Angelica* polysaccharide injection could promote the proliferation and differentiation of hemopoietic stem cell and hemopoietic progenitor, no matter if it is normal person or aplastic anemia patient that is involved, and then enrich the blood. The mechanism is possibly that it heightens the hemopoietic growth factors or has synergistic effect with them and then promotes hemopoietic function indirectly (Zhang et al., 2000; Yang et al., 2006).

Angelica polysaccharide affects hematopoiesis in different ways. There is still a lot of work to be done on control mechanism of hemopoietic factors. There is less reports on the hematopoiesis of *Angelica* polysaccharide. In this experiment, CFU-E, BFU-E and CFU-GM were cultured *in vitro*; serum containing *Angelica* polysaccharide had more notable effect on proliferation than that of the healthy serum. But when the *Angelica* polysaccharide was added to the medium, there was no obvious proliferation in the three kinds of colony. For CFU-E, BFU-E and CFU-GM, *Angelica* polysaccharide in serum has a much stronger effect than itself (Ye et al., 2001). Results showed that hematinic promoted the proliferation of bone marrow hemopoietic progenitor cells, induced secretion of cytokines at the same time. Perhaps this is one of the mechanisms on promoting the proliferation of hematopoietic progenitor cell.

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