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Effect Comparison of different formulation of Dang-Gui-Bu-Xu-Tang on myelosuppression mouse

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ABSTRACT

Objective: To compare effect of different formulation of Dang-Gui-Bu-Xu-Tang(DGBXT) on myelosuppression mouse. **Methods:** HPLC was used to measure active ingredients of two DGBXT formulations. Hemopoesic function of bone marrow was measured by hemopoietic progenitor cell culture and peipheral blood count. And hemopoietic factors in bone marrow were tested by ELISA. **Results:** The content level of astragaloside A in granule formulation was higher than that in decoction and the content ratio of active ingredient was close to 5∶1. Two DGBXD formulations could significantly improve amount of peripheral blood cells, and bone marrow cells of bone marrow suppression mice (P<0.05). DGBXD granule formulation significantly increased the colony quantity of all progenitor cell lines and amount of G_2/M and S phase cells (P<0.05). It also significantly decreased amount of G_0/G_1 phase cells in the bone marrow and was more effective (P<0.05). **Conclusions:** DGBXT decoction and the granule formulation all can improve the hematopoietic function of bone marrow suppression mouse. They can improve quantities of peripheral blood and nucleated bone marrow cells, and yield of the hematopoietic stem/progenitor cells *in vitro* colony; balance the expression of cytokine (EPO, TPO and GM-CSF) in bone marrow microenvirement. They can also facilitate hematopoietic stem/progenitor cells to enter the cell cycle. And the effect of granule formulation is more satisfactory.

1. Introduction

Human cytomegalovirus (HCMV), a member of the herpesvirus family, infects the majority of adult population. It is reported that approximately 70% of the population possess antibodies to HCMVA[1]. Acute infections by HCMV in immunocompromised individuals may lead to severe hematologic disorders, such as mononucleosis[2,3], hepatosplenomegaly[4], lymphadenopathy, thrombocytopenia[5], and hemolytic anemia[6]. Clinically, in patients undergoing bone marrow transplants, HCMV may be associated with delayed platelet engraftment, phenotypically abnormal peripheral blood leukocytes and graft failure[7], possibly through a direct viral effect on hematopoietic progenitor cells[8]. Furthermore, *in vitro* HCMV infection has shown inhibitory effect on the proliferation and colony-forming ability of hematopoietic precursors[9,10]. Dang-Gui-Bu-Xue-Tang(DGBXT), as a classical traditional Chinese medicine formula, can invigorate qi and supplement blood. It comprises of astragalus 50 g and angelica 10 g. In clinic, it is used to treat anemia and help patients recover from damage of marrow failure. As other famous ancient formulas, decoction is the main formulation. This hampers the development of traditional Chinese medicine greatly, due to uncontrollability of decoction manner, original place of crude drug, picking season, medical location of the plant and large dosage of crude drug. It is also inconvenient for patients to carry. Granule is a new formulation of the traditional Chinese medicine. It is concentrated from a single herb plant, and has been widely used in clinic. This study is to compare the efficacy between a simple combination of astragalus granule and angelica granule and traditional DGBXT decoction.

2. Materials and methods

2.1. Animal and reagent

Twenty four BALB/c inbred line male mice(8-12 w,18-22

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g) were randomly assigned to four groups: DGBXT decoction Group, DGBXT granule Group, model group and normal control group. Every group had six mice[11].

Cyclophosphamide was purchased from Shanghai Hualian Pharmaceutical Co. ^{60}Co γ was provided by Biotechnology Research Institute, Academy of Agricultural Science(Sichuan, China). DGBXT extract at 3.3 g/mL was produced according to Pharmacopoea Chinensis by Department of Traditional Chinese Medicine, Chengdu University of TCM (Sichuan, China). Chinese angelica granule and milkvetch root granule were produced by Sanjiu Modern Medicine Ltd. Ferulic acid and astragaloside Ⅳ were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

2.2. Grouping and modeling

Firstly, each mouse was irradiated by 2.0Gy ^{60}Co and than had intraperitoneal injection with cyclophosphamide (Cy) 50 mg/kg daily for three days. 24 hours after modeling, DGBXT decoction and granule were fed by intragastrical administration at 10 g/kg daily. Mice in normal control group and model group were fed by 1 mL of normal saline daily. It took seven consecutive days for administration.

2.3. Detection of ferulic acid and astragaloside Ⅳ

A waters 2695 liquid chromatograph system consisting of a quaternary pump, an autosampler and waters 2996 photodiode array detector coupled with Empower chromatographic workstation was used. The analytical column was Dia- monsil C18 reversed-phase column (4.6 mm ×150 mm, 5 μm). The mobile phase was methanol-aqueous acetic acid. The detection wavelength of the photodiode array detector was set at 254 nm (Ferulic acid and astragaloside Ⅳ); flow-rate: 1.0 mL/min.

2.4. Determination of peripheral blood cells and bone marrow nucleated cells

24 hours after the last administation, blood (10 μL) was obtained from plexus venosus behind the eyeball, and diluted with EDTA (30 μL). HS-18 type fully automated hematology analyzer (Italy) was used to detect peripheral blood cells. Single marrow cells were counted by leukocyte count assay under microscope.

2.5.The preparation of bone marrow nucleated cell suspension

24 hours after the last administration, mice were sacrificed with cervical dislocation. Femur was removed and bone marrow cells were rinsed with normal saline (1 mL) for suspension.

2.6. Hematopoietic progenitor cells culture

Bone marrow cells were plated at a concentration of 10^5/mL on semisolid methylcellulose supplemented with 30% fetal calf serum, 1% bovine serum albumin (BSA), 10^{-4} M 2-mercaptoethanol, 2 mM L-glutamine, 50 ng/mL stem cell factor, 20 ng/mL IL-3, 3 U/mL erythropoietin (EPO), 20 ng/mL rm granulocyte macrophage colony stimulating factor (GM-CSF) (PeproTech ,America), 20 ng/mL rm thrombopoient (TPO) (PeproTech, America). Plates were cultured at 37 ℃ in 5% CO_2 atmosphere. Colony-forming unit erythroid (CFU-E) were counted under inverted phase contrast microscope after 3 days of culture. Burst-forming uniterythroid (BFU-E), colony-forming unit granulocyte macrophages (CFU-GM), colony-forming unit granulocyte macrophagecolony-forming unit- megakaryocte (CFU-Meg) were counted after 7 days of culture.

2.7. Cell cycle analysis

Single bone marrow cell suspension were centrifuged (1 000 r/min, 5 min). The supernatant was removed and precipitated cells were washed twice with PBS, and then fixed with 70% cold ethanol. Before detection cells were washed again with PBS twice, and blended with PBS (100 μL), and dyed with propidium iodide (Sigma, America) staining solution (4 ℃, protection from light for 30 min). Then cell cycle was detected by flow cytometry. Data of every cell cycle phase were calculated in percentage.

2.8. Detection of bone marrow nucleated cells by sandwich ELISA

EPO, TPO and GM-CSF were detected by ELISA Kit (BioTech Sengxiong,shanghai). And OD value was measured at 492 nm.

2.9. Statistical analysis

All data were expressed as mean±SD. The one-way ANOVA was used to analyze differences among groups. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Content of ferulic acid and astragaloside in different DGBXT formulation

The results from fingerprints showed that there were significant difference in the content of ferulic acid and astragaloside Ⅳ between DGBXT decoction and granule formulation (Figure 1). The content level of astragaloside Ⅳ in granule formulation was higher than that in decoction and the content ratio of the active ingredient was near to 5∶1. As we known the weight ratio of astragalus and angelica in the traditional formula was also 5∶1 . The ratio of the ingredients in decoction was lower than that of granular formulation.

3.2. Effect on peripheral blood and bone marrow nucleated cell count

These results indicated that amount of WBC, RBC, HB, PLT and BMC in model group were significantly less than that of normal group (P<0.05). It confirmed the successful mice model of bone marrow suppression. Two DGBXD formulations could significantly improve peripheral blood and bone marrow cells of bone marrow suppression mice (P<0.05). But there was no significant difference between decoction group and granule group (Table 1).

3.3. Effect on colony yield of hematopoietic progenitor cells in vitro

It showed that DGBXT decoction significantly boosted the yield of BFU-E, CFU-E and CFU-GM cultured in vitro except CFU-Meg (P<0.05). However granule formulation significantly increased the colony quantity of all progenitor cell lines and meanwhile was more effective(P<0.05)(Table 1).

3.4. Effect on cell cycle

Table 3 showed that the quantity of G_0/G_1 phase cells was significantly increased and the amount of G_2/M and S phase cells significantly decreased in model group(P<0.05). After treatment, G_0/G_1 phase cells in the bone marrow have been significantly decreased and G_2/M and S phase cells significantly increased(P<0.05). And granule formulation was more effective than decoction(P<0.05).

3.5. Effect on TPO, EPO and GM-CSF of bone marrow stroma

Table 4 indicated that DGBXT decoction and granule formulation could significantly decreased TPO, EPO and GM-CSF content of bone marrow stroma(P<0.05). In normal conditions, there are only a few cytokines in the bone marrow stroma. Due to the damage of bone marrow stromal by modeling, cytokines were significantly increased(P <0.05). After the treatment, cytokines in the micro-environment gradually declined to normal level. And granule formulation was more effective in boosting EPO than in boosting TPO and GM-CSF.

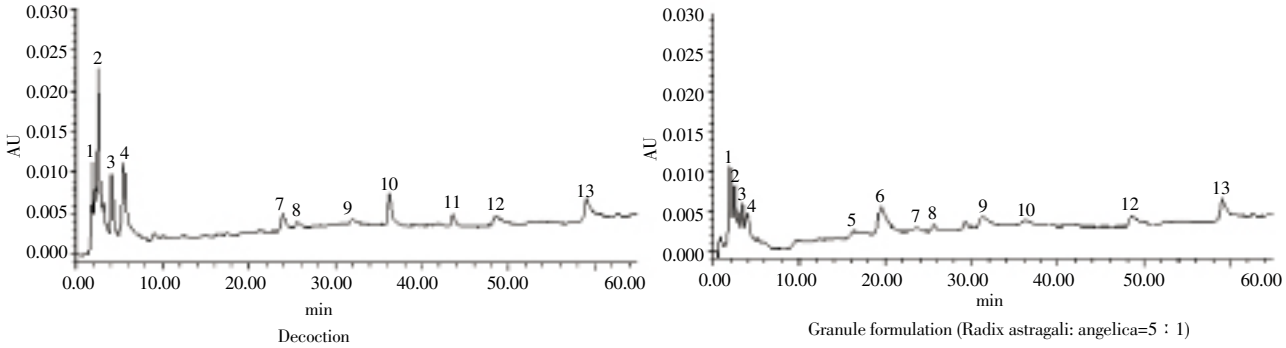

Figure 1. Finger print of ferulic acid and astragaloside Ⅳ in different DGBXT formulation.
Peak 8: ferulic, peak13: astragaloside Ⅳ.

Table 1
Effect on peripheral blood and bone marrow nucleated cell count(Mean±SD, n=6)

Group	WBC($\times10^9$/L)	RBC($\times10^{12}$/L)	HB($\times$g/L)	PLT($\times10^9$/L)	BMC($\times10^9$/L)
Normal	10.90±1.60	10.78±2.19	157.16±7.73	288.00±35.62	10.16±1.16
Model	1.05±0.42*	2.83±1.46*	101.66±2.06*	162.83±37.30*	2.00±1.26*
Decoction	7.06±2.22△	5.42±5.42△	126.66±14.26△	266.16±103.4△	3.16±1.47
Granule	8.08±6.10△	8.36±1.58△	139.33±17.82△	338.00±25.66△	9.33±2.58△

* P <0.05 *vs.* normal group; △P <0.05 *vs.* model group.

Table 2
Effect on colony yield of hematopoietic progenitor cells in vitro (Mean±SD, n=6)

Groups	BFU-E	CFU-E	CFU-Meg	CFU-GM
Normal	56.17±5.71	126.50±8.09	85.67±4.76	81.33±8.21
Model	26.50±6.25*	63.33±6.38*	36.50±4.80*	20.83±3.25*
Decoction	35.50±8.64△	75.33±7.34△	42.33±2.34	32.83±3.43△
Granule	51.83±3.87△▲	94.0±10.15△▲	61.67±8.48△▲	40.17±6.21△▲

* P <0.05 *vs.* normal group; △P <0.05 *vs.* model group; ▲P <0.05 *vs.* decoction group.

Table 3
The effect on cell cycle (Mean±SD, n=6).

Groups	G_0/G_1 phase	S phase	G_2/M phase
Normal	56.36±1.97	32.31±1.12	11.5±1.49
Model	72.21±2.10*	22.03±1.47*	6.57±0.89*
Decoction	64.88±2.01△	24.83±2.41△	8.15±0.36△
Granule	62.70±1.56△▲	28.00±2.16△▲	8.77±1.72△▲

* P <0.05 *vs.* normal group; △P <0.05 *vs.* model group; ▲P <0.05 *vs.* decoction group.

Table 4
Effect on TPO, EPO and GM-CSF of bone marrow stroma (Mean±SD, n=6).

Groups	TPO	EPO	GM-CSF
Normal	0.120±0.02	0.10±0.02	0.11±0.02
Model	0.53±0.13*	0.32±0.07*	0.54±0.13*
Decoction	0.27±0.13△	0.23±0.05△	0.25±0.11△
Granule	0.20±0.12△	0.14±0.07△▲	0.20±0.13△

*P <0.05 *vs.* normal group; △P <0.05 *vs.* model group; ▲P <0.05 *vs.* decoction group.

4. Discussion

The high infection of human cytomegalo virus can't be controlled effectively, and traditional Chinese medicine has become a new alternative treatment. It can improving immune system and can inhabit virus infection[12]. It is reported that CMV-AD169 may be inhibited or killed by GCV and *Astragalus membranaceus in vitro*[13]. Our previous studies[14,15] showed that the active ingredient, *Angelica polysaccharides* in angelica and *Astragalus astragaloside* Ⅳ in radix astragali both play an effective role in improving the colony-yield of hematopoietic progenitor/stem cells, promoting the gene expression of transcription factor GATA-1, boosting quiescent BMC to enter the cell cycle and then promoting cell proliferation. They can also promote the expression of anti-apoptotic protein Bcl-xL, inhibit apoptosis, and meanwhile influence the differentiation direction of progenitor cells. This study shows that DGBXT granule has far more rich content of ferulic acid and astragaloside Ⅳ. Besides the ratio is more closed to the weight ratio of angelica and astragalus in the traditional formula. Therefore, the granule is more effective.

In addition, some researchers[16] compared the effective content between the decoction and granule formulation using high performance liquid chromatography, and studied the efficacy of two formulations in clinic. They also hold the same option with our experimental results that the granule formulation was more effective. Therefore, the reform of Chinese medicine formulations will greatly expand the use of traditional Chinese medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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