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Pharmacological mechanism of a drug pair of *astragali* and *cinnamon* in shiquan dabu decoction

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Abstract

The pharmacological mechanism of drug pair of *astragali* and *cinnamon* in shiquan dabu decoction were studied in blood deficiency and Qi deficiency modeled mice. In Qi deficiency, the drug pair of *astragali* and *cinnamon* in shiquan dabu decoction had the ability of improving the number of white blood cells and peyer patches, and T-cell activity. It had no action in red blood cell number and phagocytic activity of peritoneal macrophage, and B-cell activity. In blood deficiency, the drug pair of *astragali* and *cinnamon* in shiquan dabu decoction had the effect of improving the hemoglobin content and T-cell activity. It did not affect the number of white blood cells and peyer patches, phagocytic activity of peritoneal macrophage and B-cell activity. In conclusion, the drug pair of *astragali* and *cinnamon* in shiquan dabu decoction has different actions, the quantity of drug pair in shiquan decoction should be changed according to the symptoms in process of diseases.

Introduction

Compound recipe is the mainly used form of Chinese medicine in the prevention and treatment of diseases (Cao et al., 2008; Song et al., 2002; Li et al., 2004). There are two main methods in the study on pharmacological mechanism of compound recipe, which are the whole and separation. The latter including of single herb, drug group and drug pair is beneficial for clarifying the mechanism, and is widely used.

Drug pair consists of two Chinese medicines. Under the guidance of theory of traditional Chinese medicine (TCM) and on the basis of drug compatibility, the two medicines are often used together in prescriptions, e.g., *ginseng* is usually combined with *astragalus*, *Gypsum* and *anemarrhena* was usually used at the same time (Chen et al., 2007).

Bazhen decoction is used in the treatment of both Qi and blood deficiency (deficiency is a description for symptoms in Chinese medicine). Qi deficiency has symptoms of pale face, dizziness, blurred vision, shortness of breath, less words, mental fatigue, and weakness (Sun, 1993; Liu et al., 2009). Blood deficiency has symptoms of yellowish face, dizziness, limbs malaise, short of breath, few words, palpitation, feeble pulse (Chen et al., 2009; You et al., 2009). More diseases, such as chronic diarrhea, chronic blood loss and so on, can lead to qi deficiency and/or blood deficiency.

Bazhen decoction is made up of 8 Chinese medicines, *angelica, ligusticum wallichii, radix paeoniae Alba, rehmannia, ginseng, atractylis ovata, tuckahoe and liquorice*. When a drug pair of *astragali* and *cinnamon* is added in bazhen decoction, another decoction Shiquan dabu is formed. Shiquan dadu decoction also had good action in the treatment of both qi and blood deficiency (Yang et al., 1986). However, the effect of this drug pair in shiquan dabu decoction is not known at present.

In this work, the pharmacology mechanism of drug pair of *astragali* and cinnamon in shiquan dabu decoction was explored.

Materials and Methods

Instrument

The UV-VIS spectrophotometer (UV-9100, Beijing Rayleigh Analytical Instruments, China), hemoglobin analyzer (Hb-1002, Shanghai Scientific Instrument Co., Ltd. China) and ELISA Microplate Reader (DG5031, East China Medical Equipment Electronics Group Co., Ltd. China) were used.

Chemical reagents

RPMI-1640 was provided by Gbico Ltd. (USA), lipopolysaccharide (LPS) were provided by the Sigma Ltd. (USA), cyclophosphamide were purchased from General Pharmaceutical Factory of Harbin Pharmaceutical Group (China), calf serum was purchased from Hangzhou Evergreen Biological Engineering Materials Co., Ltd. (China), hemoglobin determination kit was purchased from Shanghai Rongsheng Biotechnology Ltd. (China). Con A was purchased from Shanghai suolaibao Biological Technology Co. Other chemicals used in this study were of analytical grade.

Chinese medicine

All of the Chinese medicines were purchased from Guotai great dispensary (Zhenjiang, China).

Animals

Animals used for study were obtained from Jiangsu University laboratory animal Center. Kunming mice weighing 20 ± 2 g each, equal male and female, were housed in polypropylene cages after acclimatization for a period of one week in a new environment. Mice were kept at an ambient temperature and had free access to food and water.

Decoction preparations

According to the ancient medical book "Zhenti Zhenyao", shiquan dabu decoction consists of 7.8 g of each *astragali* and *cinnamon*, and 11.1 g each of other 8 Chinese medicines. In this work, Chinese medicines at the same rate aforementioned were put into an enamel pan, and water approximately 5-7 times of the medicine quantity was added. The medicines were soaked in water for 1-2 hours, and were slowly fired for an hour after boiled, then filtered, repeated once, and the filtrate were merged and concentrated into 1 g (Chinese herbal medicines) per mL by heating in water bath. In bazhen decoction, weight of 8 kinds of medicine were equal, the preparation method was same as shiquan dabu decoction.

Experimental design

Mice were randomly divided into 7 groups of each ten, normal group (A), qi deficiency group (B), blood deficiency group (C), qi deficiency group intervened with bazhen decoction (D), qi deficiency intervened with shiquan dabu decoction (E), blood deficiency intervened with bazhen decoction (F) and blood deficiency intervention group with shiquan dabu decoction (G).

Building Qi deficiency model

Each mouse of group B, D and E was administrated (i.g.) with 1 g/mL *rhubarb* soup at dose of 0.025 g/g (w/w, Chinese medicine/body weight) for 16 days. On the 8th or 9th day, mice began to lose stool, fur turned dim, dispirited and inert, reduced autonomic activities, etc., which suggested that the Qi deficiency model was successfully built. The mouse in group D and E was administrated (i.g.) with shiquan dabu decoction (0.0135 mL/g body weight) every afternoon from the 9th day to 16th, group B was treated with distilled water.

Building blood deficiency model

Each mouse of group C, F and G was intraperitoneal injection of cyclophosphamide 0.01 mg/g body weight on the 1st, 4th and 7th day to induce the blood deficiency. The mice became sluggish; fur turned dim and began to fall off, which suggested that blood deficiency model were built. Meanwhile, the mice of group F and G had been were administrated (i.g.) with Shiquan dabu decoction (0.0135 mL/g body weight) at afternoon for 8 days.

Blood assay

Blood was collected from orbital. The number of red blood cells (RBC) and white blood cells (WBC) were counted with a pool of blood counts. Hemoglobin content was measured with hemoglobin meter. Collected blood was in a water bath at 37°C for 30 min, centrifuged with 3000 rpm for 5 min, and serum was drawn and stored at -20°C for further test.

Phagocytic activity of peritoneal macrophages

Mice were intraperitoneal injection of 1% (v/v) chicken erythrocyte suspension 1 mL on next day after the last day of intervened with decoction, 40 min later, mice were died for blood collected, peritoneal cavity were opened and flushed with some physiological saline, then the peritoneal fluid were sucked out and put on a slide, was dried and fixed with methanol for 10 min, then was dyed with gimosa. The total and phagocytic macrophages were counted under microscope. The phagocytic coefficient was calculated by the amount of macrophage that devour chicken WBC divided by total amount of macrophage.

Peyer patche number

Peyer patche (PP) usually appeared white and located at the intestinal wall opposite to the mesentery. They are unequal in size, and are round or orbicular-ovate. The number of PP, diameter larger than 2 mm, was counted.

Table I

The influence of the Shiquan dabu decoction to the Qi deficiency model

Group	RBC (10^9/mm^3)	WBC (10^9/mm^3)	Hemoglobin (g/L)	PP	Phagocytic coefficient (%)	T-cell activity (A.U)	B-cell Activity (A.U.)
A	6.1 ± 0.5	9.1 ± 0.3	169.4 ± 13.4	5.5 ± 1.2	0.8 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
B	4.1 ± 0.2[a]	7.4 ± 1.3[a]	132.2 ± 9.1[a]	3.4 ± 1.2[a]	0.5 ± 0.1[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]
D	5.6 ± 0.6[b]	12.4 ± 1.9[a,b]	151.6 ± 8.0[a,b]	3.5 ± 1.0[a]	0.7 ± 0.0[a,b]	0.2 ± 0.0[b]	0.2 ± 0.0[b]
E	5.4 ± 0.2[a,b]	14.0 ± 1.4[a,b,c]	140.1 ± 9.2[a,b,c]	4.9 ± 1.5[b,c]	0.8 ± 0.1[b]	0.2 ± 0.0[a,b,d]	0.2 ± 0.0[b]

Compared with the group A, [a]p<0.01; compared with group B, [b]p<0.01; Compared with group D, [c]p<0.05, [d]p<0.01

Table II

Effect of drug pair on mice with blood deficiency

Group	RBC (10^9/mm^3)	WBC (10^3/mm3)	Hb (g/L)	PP	Phagocytic coefficient (%)	T-cell activity (A.U.)	B-cell activity (A.U.)
A	6.1 ± 0.6	9.1 ± 0.3	169.4 ± 13.4	5.5 ± 1.2	0.8 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
C	2.9 ± 0.2[a]	7.4 ± 1.2[a]	111.2 ± 10.7[a]	3.0 ± 0.8[a]	0.3 ± 0.1[a]	0.1 ± 0.1[a]	0.2 ± 0.0[a]
G	5.0 ± 0.3[a,c]	12.1 ± 1.2[a,c]	129.7 ± 12.7[a,c]	6.2 ± 1.0[c]	0.9 ± 0.1[c]	0.2 ± 0.0[b]	0.2 ± 0.0[c]
F	5.1 ± 0.3[a,c]	10.7 ± 1.9[c]	154.0 ± 11.3[a,c,d]	6.9 ± 1.8[c]	0.9 ± 0.1[a,c]	0.2 ± 0.0[c,d]	0.2 ± 0.0[c]

Compared with the group A, [a]p<0.01; compared with group B, [b]p<0.05, [c]p<0.01; Compared with group D, [d]p<0.01

Splenic T, B-cell proliferation

The spleen was taken out under sterile condition and put into a sterile petri dish, 5 mL Hanks solution was added, and then the spleen was grinded with tube bottom on a 200 copper mesh. The mixture was collected into the centrifuge tube and centrifuged at 3000 rpm for 10 min, supernatant was removed, a little of Tris-NH_4Cl was added to swell RBC. Centrifuged and removed the supernatant, added 0.5 mL PRI-1640 nutrient solution, measured the concentration of cells with trypan blue dye under microscope. The cell concentration was adjusted to 1 × 10^6 cells/mL with nutrient PRI-1640 solution. 200 µL splenic cells suspension was added to cell culture plate, then 20 µL ConA or 20 µL LPS was added. The plates were cultivated in CO_2 incubators at 37°C with 5% CO_2, 44 hours later, supernatant was disposed, 10 µL of 5 mg/mL MTT was added in darkness, and the culture was continued for 4 hours. 100 DMSO was added to each well and fully mixed. 30 min latter, the absorbance value was measured with ELISA Microplate Reader at wavelength of 570 nm.

Statistics analytical

Data was analyzed with SPSS 13.0 software. The differences among different groups were analyzed using one-way analysis of variance (ANOVA), differences were considered to be statically significant at p<0.05.

Results

The Chinese medicine theory thinks that repeated diarrhea will cause Qi deficiency. *Rhubarb* as a kind of traditional Chinese medicine was used to discharge blood stasis, remove lump, clear gastrointestinal tract. Besides, it could improve the gastrointestinal blood perfusion, enhance gastric motility and induce diarrhea. Therefore, repeated administration of rhubarb has become a method used to build qi deficiency model. The result of pharmacologic action of drug pair in shiquan dabu decoction was shown in Table I.

The number of WBC and RBC, hemoglobin content in mice with qi deficiency were significant lower than the normal (p<0.01). After treatment with bazhen or shiquan dabu decoction, they were all increased. For Group D and E, it had significant difference to group B (p<0.01), which suggested that these two decoctions had effect of improving the level of WBC, RBC and Hb in the status of Qi deficiency. For WBC and Hb, group E had significant difference to group D (p<0.05), which suggested that the drug pair of *astragali* and *cinnamon* in shiqu dabu had effect of enhancing WBC and reducing the content of Hb respectively.

The number of PP in mouse of qi deficiency was significant lower than normal (p<0.01; Table II), and was not significant increase after being treated with bazhen decoction (p>0.05). However, it increased after being treated with shiquan dabu decoction (p<0.01);

There were significant difference between bazhen and shiquan decoction ($p<0.05$), which suggested that drug pair of *astragali* and *cinnamon* had ability of enhancing PP number in shiquan dabu decoction.

Pagocytic coefficient of mice with qi deficiency were lower than normal ($p<0.01$), and increased after treatment of the two decoction ($p<0.01$). Shiquan dabu improved the pagocytic activity to normal ($p>0.05$), which suggested that the drug pair had strong effect of improving the phagocytic activity of peritoneal macrophages.

Splenic T, B-cells activity in qi deficiency mice were significant lower than normal mice ($p<0.01$). For T-cell activity, after treatment of these two decoction, they were all improved and had significant difference to group B ($p<0.01$). For group E, it had significant difference to group A and D, which suggested that the drug pair of *astragali* and *cinnamon in* shiqu dabu decoction *had* vital ability to improve the T-cell activity. For B-cell activity, bazhen and shiquan dabu decotion had ability to improve it to normal level ($p>0.05$). However, there were no significant difference between group D and E, which suggested the drug pair of *astragali* and *cinnamon* had no effect of improve the B-cell activity in shiquan dabu decoction.

In status of blood deficiency, the number of RBC and WBC and content of Hb were lower than it in normal mice, and was significant different to normal group ($p<0.01$; Table II). When mice of qi deficiency were intervened by bazhen or shiquan dabu decoction, they all increased ($p<0.01$). For Hb, it was enhanced, and had significant difference to group G ($p<0.01$), it can be inferred that the main action of drug pair *astragali* and *cinnamon* in shiquan dabu decoction was the improvement of the Hb content.

The number of PP in blood deficiency group was lower than normal ($p<0.01$), and was increased after it was administration of bazhen or shiquan dabu decoction ($p<0.01$). Compared with normal group, they were not significant difference ($p>0.05$). There were no significant difference between group G and F, which suggested that the drug pair had no action in improving PP number in shiquan dabu decoction.

Peritoneal macrophage phagocytic coefficient in blood deficiency was lower than normal group ($p<0.01$), it increased after it was administrated two kinds of decoctions ($p<0.01$). For group G, there was no significant difference to normal group ($p>0.05$). For group F, it was more than normal group and was significant different to it, which suggested that the drug pair of *astragali* and *cinnamon* had synergism with bazhen dececotin in enhancing the phagocytic activity of peritoneal macrophage.

In status of blood deficiency, splenic T, B activities were declined, and increased when was administrated with bazhen or shiquan dabu decoction ($p<0.01$). For group F, T-cell activity was significant different to group G, which suggested that the drug pair of *astragali* and *cinnamon* had effect of improving T-cell activity in shiquan dabu decoction. For B-cell activity, it had no this effect.

Discussion

It is well known that qi or/and blood deficiency can lead to decreased immune function (Wang et al., 2009). Therefore, the focus of the work is to explore the immunological mechanism of the drug pair in shiqu dabu decoction. Immune system is composed of immune organs, immune cells and immune molecules, in which immune cell is as executor of immune response.

WBC is closely related to immune. Neutrophil, as one kind of WBC, has strong phagocytosis belonging to non -specific immunity. In Human blood system, more than 95% of complement receptor one (CR1) is presented in RBC. The probability of removing the antigen-antibody-complement complexes by RBC is 500~600 times larger than it by WBC (Yang et al., 2009); therefore, RBC is also important for immune. In this work, the number of WBC and RBC in mice with qi deficiency and blood deficiency were lower than normal, which was consistent with the illness in clinic. When they were intervened by these two compound recipes, the number of WBC and RBC were all enhanced, which confirmed their pharmacological action recorded by ancient medicine book. In the status of qi deficiency, Shiquan dabu has ability in enhancing WBC, which suggested the drug pair of *astragali* and *cinnamon* has a synergistic effect with bazhen decoction. However, the drug pair had not ability in increasing the number of WBC.

PP is one kind of peripheral immune organs, it plays role in intestinal immunity. When antigen is engulfed and transferred into PP, the process including of antigen presentation, recognition, immune cell activation and proliferation take place; finally the immune function is enhanced. In addition, immune tolerance can also be induced by food antigen. Thus, the functional status of PP is associated with immune function. The number of PP will decrease when immune function decline. In this study, the number of PP in Mice with qi or blood deficiency was lower than normal, which reflected that their intestinal immunity declined. When they were administrated with bazhen decoction, the PP was improved in status of blood deficiency; however, it was not improved in status of qi deficiency. The number of PP was all increased when siquan dabu was administrated, which suggested that the drug pair of *astragali* and *cinnamon* has a strong role in the improvement of PP, through which intestinal immunity was enhanced.

T lymphocytes are composed of cytotoxic T lymphocytes (CTLs) and T helper (Th) lymphocytes. CTLs can kill virus-infected cells or tumor cells. Th lymphocytes have regulation effect in immune response. B lymphocytes can capture antigen and present to Th cells to induce immune response, it can also be differentiated to plasma cell to secret antibody to induce humoral immune response. Therefore, T and B Lymphocytes play vital role in the immune system. In this work, the B - and T-cell activity all declined in status of qi and blood deficiency, which suggested that decreased immunity was occurred. When bazhen and shiquan dabu decoction was administrated, the activity of T, B was improved. Especially for T-cell, it was highly improved, which suggested that the drug pair of *astragali* and *cinnamon* in shiqu dabu decoction has stronger activity in enhancing T-cell function. However, it has not this action for B-cell.

Peritoneal macrophages as a kind of antigen presenting cells can capture the antigen and process it into antigenic peptide, and MHC combine antigenic peptide to form complex, which is transferred on the cell membrane, which is recognized by TCR of T-cell to induce immune response. Therefore, the phagocytic activity of peritoneal macrophages is related to immunity function. In this work, bazhen and shiquan dabu decoction all had the action of the improvement of the phagocytic activity of peritoneal macrophages. However, the drug pair of *astragali* and *cinnamon* has not effect in shiquan dabu decoction.

The main chemical compositions of *astragali* were polysaccharides, saponins, flavonoids, amino acids and so on. Improvement of immune function, anti-oxidation, anti-radiation and anti-cancer have been reported. *Cinnamon* has a role in regulating immune function too. For instance, since the occurrence of asthma is related to immune dysfunction, in asthma patients, Th2 type cytokines are dominant and induce rather strong humoral immune response. Cinnamon can correct the imbalance of cytokines through down-regulating Th2 type cytokines IL-2 and IL-5 levels.

Theses documents indicated that *astragali* and *Cinnamon* had many actions in regulating immunity of body. Results in this work also suggested that the drug pair has some actions in shiquan dabu decoction. However, the mechanisms of them have not been clarified and further studies should be carried out..

In conclusion, the drug pair of *astragali* and *Cinnamon* in shiquan dabu decoction had some pharmacological actions. Chinese medicine theory thinks that medicine used should be based on symptoms. The results of this work were consistent with this theory; the drug pair *astragali* and *cinnamon* in shiquan dabu decoction has different pharmacological action in regulating body function. Therefore, the quantity of drug pair in shiquan decoction should be changed according to the symptoms in process of diseases.

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