Regular article

Study on the Changes of Chemical Constituents in Different Compatibilities of Ginseng-Prepared Rehmannia Root and Their Effects on Bone Marrow Inhibition after Chemotherapy

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Abstract:

Ginseng (G) and Prepared Rehmannia Root (PRR) are commonly used in traditional Chinese medicine for blood supplementation. This study aimed to study G and PRR with different compatibility ratios changes in chemical composition and inhibition of cyclophosphamide-induced myelosuppression. HPLC was used to determine the chemical constituents of 13 ginsenosides, 5-Hydroxymethylfurfural (5-HMF) and verbascoside in different proportions of G-PRR. Balb/C mice were injected intraperitoneally with cyclophosphamide (CTX) to induce bone marrow suppression. The effects of different proportions of G-PRR on peripheral blood, bone marrow nucleated cells, thymus and spleen index of myelosuppressed mice were analyzed. The results showed that the compatibility of G and PRR can promote the dissolution of ginsenosides, and the content of conventional ginsenosides decreased, and the content of rare ginsenosides increased. Different proportions of G-PRR increased the number of peripheral blood and bone marrow nucleated cells in cyclophosphamide-induced bone marrow suppression mice (p<0.01), increased thymus index (p<0.01), decreased spleen index (p<0.01). Different proportions of G-PRR can improve the myelosuppression induced by cyclophosphamide in mice, and the combined effect of G-PRR is better than the single decoction of G and PRR. Among them, G-PRR2:3 and G-PRR1:2 were better than the other groups. These results indicate that different proportion of G-PRR can improve bone marrow suppression, and the combined decoction of G-PRR is better than the separate Decoction in improving bone marrow suppression. This improvement may be related to the changes of the substance basis and active ingredients of G-PRR.

Keywords: Ginseng, Prepared Rehmannia Root, Myelosuppression
1. Introduction

Cancer is a leading cause of mortality worldwide and has steadily increased in the past decades\(^1\). China has become the world's second-largest cancer-prone country, with an annual increase in cancer patients accounting for about 20% of the world's total cancer patients\(^2\). Radiotherapy and chemotherapy are the main treatment methods for malignant tumors at present. However, in the course of radiotherapy and chemotherapy, radiopharmaceuticals and chemicals can not accurately act on tumor cells. When they act on normal cells, especially proliferative bone marrow hematopoietic cells, they will cause a series of toxic and side effects. Myelosuppression is one of the common side effects\(^3, 4, 5\). Severe myelosuppression can lead to a decrease in peripheral blood cell count, which may further lead to infection, anemia, hemorrhage and seriously affect chemotherapy treatment\(^6, 7\).

Ginseng is the dried root and rhizome of *Panax ginseng* C. A. Meyer. Ginsenosides are the main active ingredients of ginseng. According to previous studies, ginsenosides have the functions of improving memory function\(^8\), anti-tumor\(^9\), immune immunity\(^10\), liver protection\(^11\) and protection of cardiovascular and cerebrovascular system\(^12\). Studies have shown that ginsenoside Re and Rg1 can increase the number of white blood cells in peripheral blood, improve cyclophosphamide-induced bone marrow suppression and immune damage in mice. Moreover, the effect of ginsenoside monomer Rg1 is better than that of ginsenoside monomer Re\(^13\). Feng Cuiping et al found that ginsenoside Rh2 has protective effects on myelosuppressive mice\(^14\). Moreover, Lu Yan et al found that ginseng total saponins can promote erythroid hematopoiesis by promoting the secretion of EPO by the body and regulating the expression of corresponding receptors in hematopoietic tissues at the
transcriptional level\textsuperscript{15}).

Rehmannia Root is the tuberous root of \textit{Rehmannia glutinosa} (Gaertn.) Libosch. Ex Fisch. Et mey. It was first recorded in Shennong materia medica and listed as the top grade\textsuperscript{16}). It is one of the four Huai drugs in China. Prepared Rehmannia Root is a processed product of Rehmannia Root. It is a good Chinese herbal medicine. The word "Prepared Rehmannia Root" first appeared in the "a prescription for preparing a thousand gold coins" written by Sun Simiao, a medical expert in Tang Dynasty\textsuperscript{17}). In the southern and Northern Dynasties, there were "Lei Gong Pao Zhi Lun"\textsuperscript{18} and so on, in the Liang Dynasty, there were "Ben Cao Jing Ji Zhu"\textsuperscript{19} and so on, in the Song Dynasty, there were "Classified Materia Medica" and so on, many medical works of later dynasties recorded the cooked Rehmannia. It has the function of enriching blood, nourishing yin and replenishing essence. Modern pharmacology shows that the decoction of PRR has significant effects on the peripheral blood of mice induced by cyclophosphamide\textsuperscript{20}). Some studies have shown that PRR has a strong blood tonic effect, can promote the recovery of peripheral blood red blood cells and hemoglobin in mice with blood deficiency, and accelerate the proliferation and differentiation of bone marrow hematopoietic stem cells and progenitor cells\textsuperscript{21}).

Ginseng (G) is sweet and warm, and it has the function of invigorating vital energy. \textit{Yuanqi} is the motive force of life activities, which rooted in the \textit{kidney}, depends on the essence of the \textit{kidney} generated by the nurture of acquired Valley essence. Ginseng invigorate \textit{spleen} and \textit{lung Qi}, complex pulse and solid solution\textsuperscript{22}). Prepared Rehmannia Root (PRR) can nourish \textit{blood} and \textit{Yin}. It is an important medicinal material for nourishing \textit{blood} and treating \textit{kidney yin} deficiency and \textit{liver Yin} deficiency, and has a strong role in filling the essence\textsuperscript{23}).
G can replenish Qi to generate blood, and PRR can nourish Yin and blood to help generate Qi. Supplementary Qi and nourishing Yin and blood can promote each other, help each other to generate, enough Qi can generate blood, enough blood can help generate Qi, can assist each other. G is an important medicinal material for tonifying Qi, and its medicinal property belongs to Yang; PRR is an important medicinal material for tonifying blood, and its medicinal property belongs to Yin. G and PRR belong to Yang and Yin respectively. When G and PRR are used together, both Yin and Yang are taken into account, and both Qi and blood are supplemented. Based on the traditional efficacy of G and PRR and previous studies, we speculate that the combination of G and PRR can be used to treat chemotherapy-induced bone marrow suppression.

2. Materials and methods

2.1. Materials and reagents

The herbal medicine materials of ginseng and Prepared Rehmannia Root were purchased from the Affiliated Hospital of Changchun University of Traditional Chinese Medicine (Changchun, China). All Ginsenoside and 5-HMF, verbascoside were purchased from College of Fundamental Medical, Jilin University (Changchun, China), Shanghai Yuanye Biotechnology Co., Ltd. and Shanghai Aladdin Biochemical Technology Co., Ltd. All standards were at least 98% pure, as confirmed by HPLC. HPLC-grade acetonitrile and methanol were purchased from Fisher (USA). Cyclophosphamide was obtained from Jiangsu Shengdi Pharmaceutical Co., Ltd. (Lianyungang, Jiangsu, China). All other chemicals belong to the highest level of commercial origin.
2.2. Animals

Male Balb/c mice with body weights ranging from 18 to 22g were obtained from the Laboratory Animal Quality Testing Center of Jilin Province (Certificate No. SCXK-2016-0003) and maintained at controlled temperature (20±2°C) and humidity (50%±10%) with a 12h/12h light/dark cycle. All efforts were made to minimize the pain of animals. The animal procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of China Academy of Chinese Medical Sciences.

2.3. Grouping and modeling

After seven days of acclimatization, the mice were divided into nine groups (n=10 per group): (1) normal control group (Normal), (2) cyclophosphamide treatment group (Model), (3) Ginseng group (G), (4) Prepared Rehmannia Root (PRR), (5) Ginseng and Prepared Rehmannia Root 1:1 group (G-PRR1:1), (6) Ginseng and Prepared Rehmannia Root 1:2 group (G-PRR1:2), (7) Ginseng and Prepared Rehmannia Root 2:1 group (G-PRR2:1), (8) Ginseng and Prepared Rehmannia Root 2:3 group (G-PRR2:3), (9) Ginseng and Prepared Rehmannia Root 3:2 group (G-PRR3:2).

The mice in Groups 2-9 were intraperitoneally (i.p.) injected with a dose of CTX (100mg kg⁻¹, 0.1mL 10g⁻¹) for 3 days, and the normal group received an equal volume of saline. After CTX treatment for 24 hours, all the drug treatment groups were simultaneously administered by intragastric administration for 12 days. The mice in the control and model group were administered saline by intragastric administration (0.2mL per day).
2.4. sample preparation

Based on the data mining and statistical analysis of traditional Chinese medicine prescriptions, five different dosage ratios of G-PRR (2:1, 3:2, 1:1, 2:3, 1:2) and ginseng and rehmannia single-dose drugs were selected. Ginseng is obtained by cleaning and drying the fresh ginseng roots and rhizomes. Radix Rehmannia Preparata is made by moistening the root of Rehmannia glutinosa with clear water, placing it in a suitable steaming container, heating it with steam until it is dark and moist, taking it out, drying it to about 80% dry, cutting and drying it. Ginseng and rehmannia were scaled and all samples were extracted by water decoction. That is to take different proportions of ginseng, rehmannia single-drug, soak 10 times water for 60 minutes, boil for 60 minutes, filter out the liquid, and repeatedly cook for 3 times. The medicinal liquid was combined and concentrated. The prepared samples were G, G-PRR1:1, G-PRR1:2, G-PRR2:1, G-PRR2:3, G-PRR3:2, PRR, refrigerated for reserve.

2.5. HPLC analysis chemical composition

2.5.1 sample preparation

Samples of G, PRR and different ratios of G and PRR were converted into fresh medicinal materials of 1g and placed in 250ml conical bottles. Precision addition of methanol 100mL, weighing, sealing, put overnight, temperature 30℃ ultrasound 60minutes, and then fill the weight, filtering. 50ml filtrate was removed by a pipette and dried in an evaporating dish at 60℃. The residue was dissolved in chromatographic methanol in a 5mL volumetric bottle.

2.5.2 Chromatographic conditions

HPLC analysis of ginsenosides was carried out on an ACCHROM (S-3000) series
HPLC system (Huapu, manufactured in China). All separations were performed using an Agilent C18 column (250mm × 4.6mm, 5μm) at a column temperature of 30°C. The determination conditions were set as follows: solvent A, pure acetonitrile; and solvent B, pure water. The detection wavelength of ultraviolet lamp detector is 203nm and the flow rate is 1.0mL/min, Sample size for injection was 10μL. The gradient elution profile was programmed as follows: 18%-21% A at 0-40min, to 26% A at 42min, to 32% A at 46min, to 33% A at 66min, to 34% A at 68min, to 38% A at 73min, to 49% A at 80min. 49% A was maintained from 84 to 84min, and then changed to 51% A at 85min, to 60% A at 90min, to 65% A at 92min. 65% A was maintained from 92 to 99min, and then changed to 85% A for 104min.

HPLC analysis of Prepared Rehmannia Root was performed on an Agilent 1260 HPLC system. All separations were carried out using a Thermo C18 column (250mm × 4.6 mm, i.d., 5μm) at a column temperature of 30°C. The determination conditions were set as follows: solvent A, pure acetonitrile; and solvent B, pure water. The detection wavelength of ultraviolet lamp detector is 334nm and the flow rate is 1.0mL/min. The gradient elution profile was programmed as follows: 11% A at 0-15min, to 16% A at 16min. 16% A was maintained from 16 to 45min.

2.6. Detection of peripheral blood cells

On Day 16, blood samples were collected from the eye socket vein, and white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB), and platelets (PLTs) were counted using a cell counter. After the last drug administration, body weights of mice were measured. Blood samples were extracted from eyeballs and collected in test tubes with
ethylenediaminetetraacetic acid for further analysis.

2.7. Determinations of thymus/spleen index and bone marrow nucleated cells

After blood samples were collected, mice were euthanized by cervical vertebra dislocation and then placed in 75% ethanol for 5min twice. Immediately, the thymus gland and spleen were excised and weighed, and the thymus and spleen indices were calculated as the thymus weight/body weight and spleen weight/body weight, respectively.

Double femurs were removed under aseptic conditions, and bone marrow cells were flushed by sterile phosphate-buffered saline (PBS) to form a single cell suspension. The suspension was taken in a centrifuge tube and centrifuged at a speed of 1200r/min for 10min. And, 2mL of RBC lysis buffer was added, and it let stand for 3min after blending centrifuged for 10min at 1200r/min. The bone marrow karyocytes were removed and rinsed twice with 1mL of sterile PBS for 10min at 1200r/min and then resuspended in sterile PBS and counted by an inverted microscope.

2.8. Statistical analysis

The data were expressed as mean±standard deviation. Statistical analysis was performed by one-way analysis of variance. Statistical analysis was performed by SPSS version 19.0 (SPSS, Chicago, IL, USA) statistical software, and p<0.05 was considered statistically significant.

3. Results

3.1. Content of Ginsenoside in Different Compatibility of G-PRR

The yield of the extracts from the mixture of APIs was G (49.3%), G-PRR1:1 (63.88%), G-PRR1:2 (75.82%), G-PRR2:1 (61.2%), G-PRR2:3 (73.37%), G-PRR3:2 (64.68%) and
PRR (77.54%). High performance liquid chromatography (HPLC) was used to separate 13 ginsenosides (Rg1, Re, Rf, Rb1, Rc, Rb2, Rb3, Rd, Rk3, S-Rg3, R-Rg3, Rk1, Rg5) from ginseng decoction (Fig. 1). By plotting the integral value (X) of peak area with the quality (Y) of the standard sample in the injection, 13 standard curves were drawn and 13 linear regression equations were obtained, as shown in Table 1. According to the linear regression equation, the mass fractions of 13 ginsenosides in G decoction and G-PRR decoction were calculated, as shown in Fig. 2. Compared with G, the conventional G-PRR ginsenosides have different degrees of reduction, while the rare ginsenosides have different degrees of increase. The conventional saponin content in G was 2.088%, and the conventional ginsenoside contents of G-PRR1:2 and G-PRR2:3 were 0.743% and 1.385%, respectively. The rare saponin content in G was 0.396%, and the rare saponin contents of G-PRR1:2 and G-PRR2:3 were 2.458% and 1.946%, respectively. The Rg5 content in G-PRR1:2 was 1.604%, and the Rg5 content in G-PRR2:3 was 1.250%. The content of 13 total ginsenosides in G was determined to be 2.484%. The total amount of ginsenosides in different proportions of G-PRR was higher than that of G. Among them, G-PRR1:2 and G-PRR2:3 was higher, and the contents were 3.201% and 3.331%, respectively.

3.2. Contents of 5-HMF and Verbascoside in Different Compatibility of G-PRR

The standard curves of 5-HMF and verbascoside are shown in Table 2. The contents of 5-HMF and verbascoside are shown in Figure 3 and Figure 4. Compared with PRR group, the contents of 5-HMF in G-PRR1:1, G-PRR1:2 and G-PRR2:3 increased and showed an increasing trend. The content of G-PRR2:3 was the highest (0.115%). The content of verbascoside in Decoction with different proportion of G-PRR was higher than that in single
3.3. General status of bone marrow suppression mice

The normal group of mice had good mental state, smooth coat color, free movement, normal diet, normal growth trend, and larger body size than other groups. The model group mice had poor mental state after modeling, and the hair was dry and fluffy. They liked to shrink, move less, eat less, slow down, and lose weight. G, PRR and different proportions of G-PRR administered mice showed different degrees of improvement compared with the model group. The activity and food intake of mice in the administration group increased, and the mental state of mice in the administration group was significantly better than that in the model group. Among them, the activity and mental state of G-PRR1:2 and G-PRR2:3 were significantly better than other groups, and the improvement effect was better than other groups of mice.

3.4. Effects of different compatibility of G-PRR on peripheral blood cells induced by CTX in mice

The number of WBC, RBC, HGB and PLT from peripheral blood is shown in Fig. 5. Compared with the blank group, WBC, RBC, HGB and PLT in peripheral blood of the model group were significantly different (p<0.01), indicating that bone marrow hematopoiesis was impaired, bone marrow suppression was formed, and the model was successful. Compared with the model group mice, G, PRR and different proportions of G-PRR mice significantly increased the number of WBCs (p<0.01). And the number of PLT increased significantly (p<0.01 or p<0.05). G, PRR and different proportions of G-PRR have therapeutic effect on the increase of leukocytes and platelets in myelosuppression mice, while G and PRR have
weaker therapeutic effect than G-PRR. Compared with the model group, the number of erythrocytes in G-PRR2:3 increased significantly (P < 0.05). G-PRR2:3 has a certain therapeutic effect on the increase of erythrocytes in bone marrow inhibited mice. G, PRR and different proposals of G-PRR had no statistical difference in the amount of hemoglobin in mice. G-PRR combined decoction is better than G and PRR in peripheral blood cells, among which G-PRR1:2 and G-PRR2:3 have the best improvement effect.

3.5. Effects of different compatibility of ginseng-Radix Rehmanniae Praeparata on the BMNC counts count induced by CTX in mice

The quantity of BMNC was shown in Fig. 6. Compared with the mice in the normal group, the quantity of BMNC decreased significantly (p<0.01) in the model group. G, PRR and G-PRR all significantly increased BMNC content (p<0.01). The effect of the combination of G-PRR3:2, G-PRR2:3 and G-PRR1:2 on the bmnc of mice with bone marrow inhibition induced by cyclophosphamide was significantly better than that of the single decoction of ginseng (G) and Radix Rehmanniae Praeparata (PRR) (P < 0.01 or P < 0.05).

3.6. Effects of different compatibility of ginseng and Radix Rehmanniae Praeparata on thymus/spleen index induced by CTX

As shown in Table 3, the thymus index and spleen index of mice in model group were significantly different from those in normal group (P<0.01). Thymus index decreased, while spleen index was contrary to thymus index. G, PRR and different proportions of G-PRR can significantly increase the thymus index (P<0.01), and significantly decreased the spleen index (p < 0.01). The efficacy of the mixed decoction groups were better than that of the single decoction groups, and the G-PRR1:2 and G-PRR2:3 groups were the best.
4. Discussion

Traditional Chinese medicine compatibility prescription is an important form of traditional Chinese medicine\(^{25}\). The process of decoction and preparation of traditional Chinese medicine after compatibility is complex, which will produce physical and chemical changes. Because of the coexistence of many components, it may produce changes in PH, which may change the content of some chemical components and may produce new compounds\(^{26, 27}\). The results showed that the total saponin content of G-PRR in different proportions was higher than G, indicating that PRR can promote the dissolution of G on ginsenosides. In the process of ginseng processing, temperature will affect the conversion of ginsenosides into rare ginsenosides\(^{28, 29}\). Studies have shown that the dissolution rate of ginsenosides can be increased by decocting ginseng in water with other traditional Chinese medicines\(^{30}\). Studies have shown that ginseng is decocted in water with other traditional Chinese medicines, the acidic components in the medicinal materials will decrease the pH value of the solution and lead the sugar chain of ginsenosides to break and transform. Ginsenosides can be transformed from saponins with larger molecular weight to secondary saponins with smaller molecular weight or rare saponins with stronger biological activity\(^{31}\). The study also found that the compatibility of G and PRR promoted the conversion of traditional ginsenosides to rare ginsenosides. Among the five different proportions of G-PRR, triol-type saponins Re were transformed into rare saponins Rk3 in varying degrees. Among them, the increase of G-PRR1:2 is the largest. The contents of Re and Rk3 in G were 0.287% and 0.057%, respectively, and the contents of Re and RK3 in G-PRR1:2 were 0.054% and 0.340%, respectively. The increase of G-PRR2:3 was second only to that of G-PRR1:2. The
contents of Re and Rk3 were 0.166% and 0.228%, respectively. Diol-type saponins Rb1, Rc, Rb2 and Rb3 were transformed into rare saponins Rk1, Rg3 and Rg5 in varying degrees. Among them, G-PRR1:2 increased the most, and the contents of Rk1 and Rg5 were 0.219% and 1.604%, respectively. The increase of G-PRR2:3 was second only to that of G-PRR1:2. The contents of Rk1 and Rg5 were 0.156% and 1.250% respectively. Ginsenoside is the main active component of Ginseng. It has been reported that total saponins of ginseng can promote the recovery of erythrocyte, hemoglobin and bone marrow cells in peripheral blood of irradiated mice, and promote the proliferation of erythroid progenitor cells and granulocyte hematopoietic progenitor cells (CFU-GM)\(^{32, 33}\). It has been reported that many monomer saponins of Ginseng can improve hematopoietic function of cyclophosphamide-induced bone marrow suppression\(^{34, 35, 36}\). Therefore, we infer that these active ingredients have inhibitory effects on bone marrow suppression.

5-HMF and verbascoside are quantitative indicators for the quality control of PRR\(^ {37, 38}\). In our study, G-PRR with different proportion significantly increased the content of verbascoside. At the same time, when the proportion of PRR was greater than G, the content of 5-HMF increased in varying degrees compared with the content of PRR in single decoction. These active ingredients may contribute to the overall pharmacological action.

Cyclophosphamide is a bifunctional alkylator and cell cycle nonspecific chemotherapeutic drug. The phosphophthalazine nitrogen mustard is metabolized by cyclophosphamide, which has cytotoxic effect on tumor cells, and can also cause damage to bone marrow cells with active proliferation\(^{39, 40}\). Cyclophosphamide can cause severe bone marrow suppression in large doses or long-term use\(^ {41}\). Therefore, in immunotoxicology,
cyclophosphamide is a commonly used drug in the preparation of immunosuppressive models\(^4^2\). In this study, the model of mice with myelosuppression was established by i.p. injection of CTX.

Bone marrow is the main hematopoietic organ of human body. Hematopoietic stem cells are the predecessors of hematopoietic progenitor cells. Red blood cells, granulocytes and megakaryocytes differentiate and develop into red blood cells, white blood cells and platelets in peripheral blood\(^4^3\). Therefore, the number of peripheral blood cells is one of the important indicators to detect the hematopoietic function of bone marrow. Myelosuppression is manifested as hematopoiesis disorder, in which leukocyte is the most influential factor, followed by platelet\(^4^4\). This study found that cyclophosphamide could significantly reduce the number of peripheral blood cells, G, G-PRR and different proportion of G-PRR group could significantly improve the white blood cells and platelets in peripheral blood, and different proportions of G-PRR could improve the white blood cells and platelets in peripheral blood better than single decoction, of which G-PRR2:3 and G-PRR1:2 group had the best effect. Bone marrow nucleated cells refer to the cells containing nuclei in bone marrow cells besides mature red blood cells and platelets, including granulocyte lines, giant cell lines, monocyte lines and lymphocyte lines. Proliferation, apoptosis and maturation release of divisive cells in bone marrow nucleated cells determine the maintenance and dynamic balance of their number, which can directly reflect the proliferation status and ability of bone marrow hematopoietic cells\(^4^5\), so it is also an important indicator of bone marrow hematopoietic function. This study found that cyclophosphamide can significantly reduce the number of BMNCs, G, PRR and different proportion of G-PRR group can
significantly improve BMNCs, different proportion of G-PRR to improve the efficacy of BMNCs is better than single decoction, of which G-PRR2:3 group is the best. These results suggest that G and PRR can promote the recovery of hematopoietic function of bone marrow and differentiate into peripheral blood cells and bone marrow nucleated cells. Moreover, the combined use of G and PRR had better effect, and G-PRR2:3 and G-PRR1:2 had the best effect. It shows that G-PRR combined with decoction has a strong therapeutic effect on cyclophosphamide induced myelosuppression.

Thymus, spleen and bone marrow are important immune organs in animals. Thymus is the central immune organ of T cell differentiation and maturation, which is related to fine immunity. The spleen is the largest peripheral immune organ in the body. It is one of the places where allergic lymphocytes and antibodies are produced, and it can remove foreign bodies such as blood cells and bacteria that are disabled and senile by itself\(^{46}\).

Cyclophosphamide can lead to thinning of thymic cortex, unclear demarcation between cortex and medulla, lymphocytopenia and other pathological changes in mice, reducing thymic index. It can also cause splenic hemorrhage, fibrous tissue proliferation and other pathological changes, increase the amount of spleen index\(^ {47}\). In this experiment, the thymus of mice modeled by cyclophosphamide decreased, the thymus index decreased, the spleen produced compensatory hyperplasia, and the spleen index increased. G, PRR and different proportion of G-PRR can promote the recovery of thymus and spleen, increase thymus index and decrease spleen index. In addition, the combination of G and PRR can better promote the recovery of thymus and spleen, and G-PRR2:3 and G-PRR1:2 has the best recovery effect. It may be that the combination of G-PRR can restore part of the hematopoietic function of
mouse bone marrow and promote the recovery of immune organs.

5. Conclusion

To sum up, this experiment compared the saponins of G and PRR before and after their compatibility with each other in different proportions and their effects on improving bone marrow suppression. The results showed that after compatibility with Rehmannia glutinosa, the total saponins in ginseng increased, the conventional saponins decreased to varying degrees, and the total amount of rare ginsenosides increased. And different proportions of G-PRR have an effect on the improvement of myelosuppression, and the combined decoction of G and PRR is better than the improvement of myelosuppression by their respective single decoction. This improvement may be related to the material basis of G-PRR and changes in active ingredients. The results of this study are of great significance for the development of G-LLP as a preventive and therapeutic agent for human myelosuppression. In order to further study the pharmacodynamic basis of G-PRR, it provides a new idea for clinical prescriptions of Chinese medicine.

Conflict of Interest The authors declare no conflict of interest.
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### Table 1

Regression equation

| Ginsenoside | Regression equation | $R^2$ | Linear range/(μg*mL$^{-1}$) |
|-------------|---------------------|-------|-----------------------------|
| Rg1         | $Y = 2.605X - 10.796$ | 0.9996 | 31.406-502.50               |
| Re          | $Y = 2.586X - 8.9447$  | 0.9999 | 31.250-500.00               |
| Rf          | $Y = 3.3206X - 1.3422$ | 0.9997 | 7.8906-126.25               |
| Rb1         | $Y = 2.4026X - 12.511$ | 0.9999 | 31.250-500.00               |
| Rc          | $Y = 2.7555X - 9.5525$ | 1      | 31.406-500.00               |
| Rb2         | $Y = 2.6656X - 10.301$ | 0.9999 | 15.625-250.00               |
| Rb3         | $Y = 1.9131X + 1.6097$ | 0.9998 | 7.8125-125.00               |
| Rd          | $Y = 3.2336X - 7.3088$ | 0.9999 | 15.703-251.25               |
| Rk3         | $Y = 1.0206X - 2.8449$ | 0.9993 | 7.8125-125.00               |
| S-Rg3       | $Y = 3.2032X - 3.1435$ | 0.9994 | 7.8906-126.25               |
| R-Rg3       | $Y = 3.5662X - 2.2585$ | 0.9993 | 7.8906-126.25               |
| Rk1         | $Y = 3.7933X + 5.9619$ | 0.9998 | 7.8125-125.00               |
| Rg5         | $Y = 0.8252X - 12.021$ | 1      | 62.813-1005.0               |
Fig. 1. The remarkable ginsenoside profile differences between G and PRR. (A) Calibration curve; (B) G; (C) G-PRR 1:1; (D) G-PRR 1:2; (E) G-PRR 2:1; (F) G-PRR 2:3; (G) G-PRR 3:2; Peak numbers: 1, Rg1; 2, Re; 3, Rf; 4, Rb1; 5, Rc; 6, Rb2; 7, Rb3; 8, Rd; 9, Rk3; 10, 20(S)-Rg3; 11, 20(R)-Rg3; 12, Rk1; 13, Rg5.
**Fig. 2.** The chemical content changes between the combined decoction and the separated decoction. The values presented are the mean ± SD (n=3 in each group). --p < 0.01, vs G (reduce); ++p < 0.01, vs G (increase).
Table 2

Regression equation

| Component    | Regression equation | $R^2$ | Linear range/(μg/mL$^{-1}$) |
|--------------|---------------------|-------|-----------------------------|
| 5-HMF        | $Y=0.2912X+0.4042$  | 0.9999| 7.8516-125.63               |
| verbascoside | $Y=6.8212X-5.1833$  | 0.9996| 7.8125-125.00               |
Fig. 3. HPLC chromatograms showing the chemical composition of G and G-PRR. (A) Calibration curve; (B) G; (C) G-PRR 1:1; (D) G-PRR 1:2; (E) G-PRR 2:1; (F) G-PRR 2:3; (G) G-PRR 3:2; Peak numbers: 1, 5-HMF; 2, Verbascoside.
Fig. 4. Contents of 5-HMF and Verbascoside in G-PRR with Different Compatibilities. The values presented are the mean ± SD (n=3 in each group). --p < 0.01, vs PRR (reduce); ++p < 0.01, vs PRR (increase).
Fig. 5. Effect of G-PRR on peripheral blood cells in Cy-induced mice. (A) WBC, (B) RBC, (C) HGB, (D) PLT. Data are expressed as mean±SD. #P<0.05, ##P<0.01, vs normal; *P<0.05, **P<0.01, vs model. G-PRR, Ginseng Radix Rehmanniae Preparata herb pair; HGB, hemoglobin; PLT, platelet; RBC, red blood cell; WBC, white blood cell.
Fig. 6. Effects of different compatibility of Ginseng and Prepared Rehmannia Root on the BMNC counts count induced by CTX in mice. Data are expressed as mean±SD(n=6).

##p<0.01 as compared with the normal group. **p<0.01 as compared with the model group.

a$p<0.05$ or aa$p<0.01$ as compared with the G group, b$p<0.05$ or bb$p<0.01$ as compared with the PRR group.
Table 3

Effects of different compatibility of Ginseng and Prepared Rehmannia Root on thymus/spleen index induced by CTX

| Groups       | Thymus index (mg/g) | Spleen index (mg/g) |
|--------------|---------------------|---------------------|
| Normal       | 1.47±0.11           | 4.09±0.67           |
| Model        | 0.48±0.09##         | 6.43±0.67##         |
| G            | 0.75±0.11**         | 5.25±0.81**         |
| PRR          | 0.72±0.12**         | 5.65±0.68**         |
| G-PRR1:1     | 0.81±0.11**         | 5.25±0.76**         |
| G-PRR1:2     | 0.92±0.14**         | 5.15±0.95**         |
| G-PRR2:1     | 0.73±0.13**         | 5.34±0.86**         |
| G-PRR2:3     | 0.98±0.10**         | 5.13±0.78**         |
| G-PRR3:2     | 0.76±0.14**         | 5.30±0.69**         |

Data are expressed as mean±SD (n=10)

##p < 0.01 as compared with the normal group. **p<0.01 as compared with the model group. CTX, cyclophosphamide; SD, standard deviation