The Effects of Huangqi Dihuang Pill on Immune Function of Immunosuppression Mice

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Abstract. Objective: Study the effects of Huangqi Dihuang pill(HDP) on immune function of immunosuppression mice. Methods: The mouse immunosuppression model was established by intraperitoneal injection of cyclophosphamide, intraperitoneal injection of chicken erythrocytes, intramuscular injection PHA, Then intraperitoneal injection of chicken erythrocytes and hennessy to detect the effects of Huangqi Dihuang pill on hemolysin, hemolytic plaque, lymphocyte transformation and phagocytosis of macrophages. Results: Huangqi Dihuang pill could improve the function of hemolysin, hemolytic plaque, lymphocyte transformation and macrophage phagocytosis of immunosuppression mice significantly (P<0.01). Conclusion: Huangqi Dihuang pill could improve the immune function of immunosuppression mice and is a potential immunomodulator.

Introduction

Immune system is an important system that body performs immune response and immune function. It has the function of identifying and eliminating the antigenic foreign body, coordinating with other systems of the body, and jointly maintaining the stability and physiological balance of the body. The imbalance and dysfunction of the immune system can lead to a range of related diseases, such as chronic inflammatory bowel disease (IBD)[1], autism (ASD)[2]. The immune system is classified into non-specific immunity and specific immunity, and specific immunity is divided into humoral immunity and cellular immunity. Subsequently, some scholars proposed the mucosal immune system and studied it as a relatively independent immune system[3]. Recent studies have shown that plant polysaccharides [4], flavonoids [5], glycosides [6] and other components have extensive immunological functions. Both astragalus and rhubarb are also recorded in the shennong grass sutra, which is also recorded in other medical literatures, and both contain polysaccharides, flavonoids, glycosides and other components. The preliminary study of the experimental team also showed that both the rhubarb and astragalus had enhanced immunity[7-8]. Huangqi dihuang pills are mainly composed of astragalus and rhubarb. Therefore, in this experiment, we discussed whether huangqi dihuang pills had an effect on immune function of the immunocompromised mice induced by humoral immunity from three aspects of humoral immunity (hemolysin, hemolytic plaque), cellular immunity (lymphocyte conversion rate) and non-specific immunity (macrophage phagocytosis).
Experimental Materials

Organization of the Text

Drug

Huangqi dihuang pills (HDP), provided by Henan University of Traditional Chinese Medicine; Shiitake polysaccharide tablets (SPT), provided by Kaifeng Pharmaceutical (Group) Co., LTD., batch no. 16010102;
Plant hemagglutinin, Shanghai Xinguowei Medical Equipment Co., LTD. Batch number 20155902;
Injection cyclophosphamide: Jiangsu Shengdi Pharmaceutical Co., LTD. Batch no. 16021625;
10% complement preparation: Carotid artery taking blood from guinea pigs, centrifugally take the supernatant liquid and add it to the red blood cell. Make the ratio of serum to red blood cells was 1:0.2 (V/V), again after the centrifugal drain on the liquid, with 0.9% sodium chloride injection diluted into 1:10, 4 °C refrigerator storage.

Animals and Experimental Apparatus


Experimental Method[9,10,11]

Organization of the Text

The Effect of HDP on Hemolysin and Hemolytic Plaque Formation in Immunosuppression Mice

Kunming mice 72, randomly divided into 6 groups, each group 12, which were blank group, model group, positive control group, Huangqi dihuang pills large, medium and small dose group. In addition to the blank group, the immunosuppressive model of cyclophosphamide was induced. Each group was injected with 100 mg/kg of intraperitoneal injection every day. In addition to the model group, the Huangqi dihuang pills large dose, medium dose and small dose suspension (5g/kg, 2.5g/kg, 1.25g/kg) and the mixed suspension of the mushroom polysaccharide tablets (MPT) (15mg/kg) was continuously administered respectively, continuous 7d. In the 1d of administered, intraperitoneal injection of 5% chicken erythrocyte physiological saline solution 0.2ml each. After fasting for 12h on the 7th day, giving the medicine, after 2h of giving the medicine, the blood was taken from the eyeball and the serum was centrifuged. 1:10 diluted serum with physiological saline, take 1ml diluent, 0.5ml 5% chicken red blood cell suspension liquid and, 0.5 ml10 % complement blending, 37°C incubation 30 min, end in the ice water reaction, centrifugal again. In addition, a model tube with no serum was set as control. Set the zero with the model tube to determine the formation of hemolysin in each group in 540nm of Spectrophotometer.
After taking blood, the mice were executed, dissected and extracted spleen. The spleen cells of the two mice were put together with homogenized homogenate, and the splenic cell suspension was adjusted. The number of spleen cells was 5 \times 10^6 /ml. 0.5ml of splenic cell suspension was taken, with 0.5 ml2 % chicken red blood cell suspension and 0.5ml10% complement. Setting up without complement with physiological saline added with volume as a model of care, 37°C for 1 h incubation, centrifugal. Set the zero with the model tube to determine the formation of hemolytic plaque in each group in 413 nm of Spectrophotometer.

**The Effect of HDP on Lymphocyte Conversion Rate in Immunosuppression Mice**

Grouping, molding, and drug delivery made as above. In addition to the blank group, the groups were injected with PHA(10mg/kg,0.1ml/10g) in the first, second and third days of the administration. After fasting for 12h on the 7th day, giving the medicine, after 2h of giving the medicine. Cut off the tail to take blood, smear, with Switzerland’s dye solution dyed. At high magnification, the percentage of lymphocyte transformation was calculated.

Lymphocyte conversion percentage = transformed lymphocytes/transformed lymphocytes + unconverted lymphocytes *100%.

**The Effect of HDP on Phagocytic Percentage and Phagocytic Index in Immunosuppression Mice**

Grouping, molding, and drug delivery made as above. For the 2h later after last time of administered, each mice intraperitoneal injection of 0.5ml5% chicken red blood cell suspension. After 4 hours, the mice were executed, and the intraperitoneal was injected with 2.5ml of henna liquid. The abdominal skin of the mouse was cut, and a small hole was cut in the peritoneum, and extract the abdominal fluid 2ml in the test tube. Mix evenly and absorb a small amount of abdominal fluid onto the slide, make the droplet size is about 1.5*2 cm. 37°C incubation for 30 minutes, rushed to adherent cells with physiological saline, with Switzerland’s dye solution dyed. Drying, under the microscope, the macrophages erythrocytes chicken red blood cells were observed, and the percentage of phagocytic and phagocytic index were calculated.

Phagocytosis percentage =The number of macrophages in 100 macrophages that eat chicken erythrocytes /100;

Phagocytic index = the total number of red blood cells consumed by 100 macrophages/100.

**Experimental Results**

**The Effect of HDP on Hemolysin, Hemolytic Plaque Formation and Lymphocyte Conversion rate in Immunosuppression mice**

As we can see from Table 1: Compared with the blank group, the formation of hemolysin and hemolytic plaque in the model group decreased significantly, and the conversion rate of lymphocytes decreased significantly (P<0.01). Group compared with model group, MPT group, HDP large, medium and small dose group could significantly increase the formation of hemolysin, hemolytic plaque and the conversion rate of lymphocytes (P<0.01). Moreover, the effect was most obvious in the HDP medium and low dose group.
Table 1. Effect of HDP on hemolysin, hemolytic plaque formation and lymphocyte conversion rate in immunosuppression mice (x±s, N=12).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>hemolysin</th>
<th>hemolytic plaque</th>
<th>lymphocyte conversion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>-</td>
<td>0.13±0.01</td>
<td>0.35±0.0027</td>
<td>44.8±2.32</td>
</tr>
<tr>
<td>Model</td>
<td>-</td>
<td>0.069±0.12##</td>
<td>0.251±0.014##</td>
<td>34.9±2.52##</td>
</tr>
<tr>
<td>MPT group</td>
<td>0.015</td>
<td>0.127±0.015**</td>
<td>0.374±0.016**</td>
<td>62.1±2.76##</td>
</tr>
<tr>
<td>HDP large dose group</td>
<td>5</td>
<td>0.128±0.008**</td>
<td>0.361±0.021**</td>
<td>57.2±1.28##</td>
</tr>
<tr>
<td>HDP medium dose group</td>
<td>2.5</td>
<td>0.134±0.012**</td>
<td>0.365±0.018**</td>
<td>61.4±3.00##</td>
</tr>
<tr>
<td>HDP small dose group</td>
<td>1.25</td>
<td>0.132±0.011**</td>
<td>0.378±0.015**</td>
<td>60.1±2.40##</td>
</tr>
</tbody>
</table>

#Compared with blank group P <0.05; ##Compared with blank group P <0.01.
*Compared with model group P <0.05; **Compared with model group P <0.01.

The Effect of HDP on Phagocytic Percentage and Phagocytic Index in Immunosuppression Mice

Table 2. Effect of HDP on phagocytic percentage and phagocytic index in immunosuppression mice (x±s, N=12).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>phagocytic percentage</th>
<th>phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>-</td>
<td>43.9±2.7</td>
<td>0.598±0.07</td>
</tr>
<tr>
<td>Model</td>
<td>-</td>
<td>36.4±1.7##</td>
<td>0.463±0.075##</td>
</tr>
<tr>
<td>MPT group</td>
<td>15</td>
<td>51.3±3.7**</td>
<td>0.69±0.083**</td>
</tr>
<tr>
<td>HDP large dose group</td>
<td>5</td>
<td>47.6±3.1**</td>
<td>0.612±0.080**</td>
</tr>
<tr>
<td>HDP medium dose group</td>
<td>2.5</td>
<td>53.9±2.8**</td>
<td>0.689±0.094**</td>
</tr>
<tr>
<td>HDP small dose group</td>
<td>1.25</td>
<td>50.6±4.0**</td>
<td>0.639±0.103**</td>
</tr>
</tbody>
</table>

#Compared with blank group P <0.05; ##Compared with blank group P <0.01.
*Compared with model group P <0.05; **Compared with model group P <0.01.

As we can see from Table 1: Compared with the blank group, the phagocytic percentage and phagocytic index of the mouse in the model group decreased significantly (P<0.01). Compared with the model group, the phagocytic percentage and phagocytic index of chicken erythrocyte were significantly increased in each group of mice (P<0.01).
Summary

Immunization is an important pathophysiological process of the body, by eliminating "feiji" for the protection of the body, the immune response is too weak or defects can lead to autoimmune defects related diseases [12]. It is the key to maintain the normal life of the body by using the external material to regulate the body properly and effectively. Astragalus has the function of promoting metabolism, enhancing and regulating body immunity, enhancing myocardial systolic function, lowering blood sugar and fatigue, etc; Rhubarb has many kinds of pharmacological effects, such as enhanced immunity, cardiovascular and cerebrovascular diseases, neuroprotection, anti-diabetes and its complications. Based on the components of the immune system, the experiment from nonspecific immune (macrophage phagocytosis) and specific immune, humoral immunity and cellular immunity) two aspects discusses the immunoregulatory effects of huangqi Dihuang pill.

The immune organ is the place where immune cells take place, differentiate and proliferate, and it is also the place where the immune response takes place. The spleen is the peripheral immune organ, which is the site of immune cell settlement and immune response, and the number of hemolytic plaques reflects the humoral immune function of the body. Hemolysin is the antibody produced by antigen stimulation of B lymphocytes and is also an important index for evaluating humoral immunity. Lymphocytes are mainly distributed in peripheral immune organs and blood, and are important cell types that constitute the body’s immune system. The rate of lymphocyte conversion is an indirect reflection of cell proliferation and is an important indicator of cellular immunity. Macrophages are an important non-specific immune effector cell, and the phagocytic percentage and phagocytic index can reflect the strength of phagocytic ability. Macrophages can effectively defend against endogenous or exogenous pathogens and become the first line of defense against pathogenic microorganism infection [13,14]. Hemolysin and hemolytic plaque, lymphocyte conversion rate, phagocytic percentage and phagocytic index can partly reflect the humoral immunity, cellular immunity and nonspecific immunity. The experimental results show that huangqi dihuang pills could adjust the immune function of cyclophosphamide lower immune mice by promoting the formation of hemolysin and hemolysis void and promote the lymphocyte conversion rate, strengthen the devouring percentage and index of macrophage. Compared with lentinus polysaccharide, huangqi dihuang pill has certain advantages in the improvement of humoral immunity and non-specific immunity.

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References


