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EFFECTS OF CHINESE HERBAL RECIPES ON IMMUNITY IN IMMUNOSUPPRESSIVE MICE

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Abstract

The Chinese herbal formula consisting of *Astragalus membranaceus*, *Epimedium brevicornum*, *Paeoniae Alba Radix* and *Radix Ophiopogonis* in proper proportions were adopted in order to investigate the immunoenhancing properties of the herbal formula. Fifty ICR mice were randomly divided into 5 groups (NS，NS+Hy，L+Hy，M+Hy，H+Hy). The mice in hydrocortisone (Hy) groups were injected with hydrocortisone i.p. to induce the immunosuppressive condition. The mice in group NS were administered with normal saline as controls. The mice in groups NS+Hy，L+Hy，M+Hy，H+Hy were administered with normal saline, low, moderate and high dose of the herbal prescription respectively by gavage for 6 days. The level of serum hemolysin, the function of antibody function cell（AFC）and $CD4^+/CD8^+$ T cell ratio were measured at the end of experiments. The results showed that the level of serum hemolysin, the function of AFC and $CD4^+/CD8^+$ T cell ratio in L+Hy，M+Hy，H+Hy groups increased significantly compared with those in NS or NS+Hy groups. These results indicate that Chinese herbal medicine prescription can enhance humoral immunity and cellular immune function of the immunosuppressive mouse.

Key words: Chinese herbal medicine; immunosuppression; serum hemolysin; $CD4^+/CD8^+$T cell ratio

Introduction

With the development of molecular biology and immunopharmacology, Chinese herbal medicine immunology has become an important branch of modern medicine. In recent years, immunomodulating properties and disease resistance of Chinese herbal medicines were investigated with a series of achievements (Ma, 2007; Chi and Wu, 2004). In this experiment, the study of the effects of the herbal medicine formula composed of *Astragalus membranaceus*, *Epimedium brevicornum*, *Paeoniae Alba Radix* and *Radix Ophiopogonis* was carried out through measuring the serum hemolysin level, antibody function cells and the peripheral blood T-lymphocyte subsets of the immunosuppressive mice. The results of this study provide not only a theoretical basis for clinical application, but also a scientific basis for finding new effective immunoregulators of Chinese herbal medicine.

Materials and Methods

Herbal decoction

The herbs *Astragalus membranaceus*, *Epimedium brevicornum*, *Paeoniae Alba Radix* and *Radix Ophiopogonis*, purchased from an herbal shop in Baoding City and authenticated by Hebei Provincial Bureau of Herbal Medicine, were mixed, minced, soaked in water for an hour, and boiled for an hour. After that the supernatant was collected. The above procedure was repeated and the supernatant was collected. The all supernatant was mixed together, and simmered to a desired volume (1g/ml).

Animals

ICR mice of 20-22g body weight were provided by Beijing Experimental Animal Center, conventionally reared, fed and watered ad lib with a light cycle of 12h each day. All the experimental animals were treated in accordance with the guidelines of the Chinese Council for Animal Care.

Reagents

Hydrocortisone (Hy) was purchased from Tianjin Jiaozuo Pharmaceutical Co., Ltd. CD4-FITC and CD8-PE monoclonal antibodies were purchased from Pharmingen, USA .

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The influence on the level of serum hemolysin and the function of AFC in immunosuppressive mice: Experimental design and assignment

Fifty ICR mice were randomly divided into five groups, normal saline control group (NS), Hydrocortisone plus normal saline group (NS+Hy), Hydrocortisone plus low-dose herb group (L+Hy), Hydrocortisone plus middle-dose herb group (M+Hy), Hydrocortisone plus high-dose herb group (H+Hy). The immunosuppressive mice model was established by intraperitoneal injection (i.p.) of hydrocortisone (Shi et al,1996). All mice were administered with the herbal decoction by gavage for six days consecutively（Table 1). Each mouse in each group was given 20g/L sheep red blood cells (SRBC) 0.2mL by intraperitoneal injection 3 days post Chinese herbal recipe treatment. The level of serum hemolysin and the function of AFC were measured 6 days post Chinese herbal treatment.

Table 1: Experimental design and assignment

Groups	Sample (n)	Dosage (ml)
NS	10	0.4ml
NS+Hy	10	0.4ml
L+Hy	10	0.2ml
M+Hy	10	0.4ml
H+Hy	10	0.6ml

The level of serum hemolysin in mice

The blood samples from orbit of mice were collected and the sera were isolated. The level of serum hemolysin was measured according to the literature (Shan and Pu, 2005). The level of serum hemolysin was measured with hemolytic concentration (HC_{50}), HC_{50}=sample absorption /bbsorption at HC_{50} of SRBC × diluted time.

The function of AFC in spleen

At the end of the experiment, the mice were sacrificed and the spleen were carefully sheared and resuspended into 2.0×10^6 cell/mL solutions. Then splenocyte suspension, 2g/L SRBC and 10% complement were added at 1:1:1 ratio to measure the level of antibody function cell (AFC). The reaction mix was then shaken thoroughly, incubated at 37°C for 1 hour, centrifuged at 3000 r/min for 10 minutes. Then the supernatant was collected and the OD value measured at 413 nm. The splenocyte suspension was replaced by normal saline as blank.

The influence of $CD4^+/CD8^+$T cell ratio in peripheral blood

Experimental design and assignment were described in section 2.4.1. Each mouse was given Chinese herbal medicine once a day for 6 successive days. Twenty-four hours after the last administration, 0.1ml blood was collected from orbit of mice, anticoagulated with 50U/mL heparin and used to measure the ratio of T-lymphocyte subsets ($CD4^+/CD8^+$). $CD4^+/CD8^+$T cell ratio was measured by Flow cytometry as descripted by Liblau et al (1995).

Data analysis

The data were taken as mean and standard deviation, and analyzed with SPSS11.0. The data were assessed by Student's *t*-test.

Results

The level of serum hemolysin in immunosuppressive mice

The level of serum hemolysin was significantly decreased in the Hy group as compared to saline group. The levels of serum hemolysin in all of the three Chinese herbal groups were significantly higher than that of NS+Hy. Especially, the level of serum hemolysin in the high dose herbal group was significantly higher compared with that in NS+Hy group ($P<0.01$) (See Table 2).

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Table 2: Effects of Chinese herbal medicine on the level of serum hemolysin in immunosuppressive mice

Groups	Dosage(mL/mouse)	Sample (n)	HC_{50}
NS	0.4	10	18.13±5.40
NS+Hy	0.4	10	15.44±5.37
L+Hy	0.2	10	20.35±4.19*
M+Hy	0.4	10	23.05±5.59**a
H+Hy	0.6	10	25.08±6.54**A

Note:Compared with NS+HY control group,**(P<0.01);* (P<0.05); compared with NS control group, A (P<0.01), a (P<0.05)

The function of AFC in immunosuppressive mice

The function of AFC was decreased significantly by Hy administration. The values of AFC in Chinese herbal groups were significantly higher than that of NS+Hy (P<0.05). Especially, AFC in middle and high dose groups were significantly different compared with that of NS group (P<0.01) (See Table 3).

Table 3: Effect of Chinese herbal medicine on the function of AFC in immunosuppressive mice

Groups	Dosage(mL/mouse)	Sample (n)	A_{413}
NS	0.4	10	0.099±0.023
NS+Hy	0.4	10	0.069±0.030a
L+Hy	0.2	10	0.086±0.025
M+Hy	0.4	10	0.101±0.016**
H+Hy	0.6	10	0.106±0.026**

Note: Compared with NS+HY control group,**(P<0.01);* (P<0.05); compared with NS control group, A (P<0.01), a (P<0.05)

Changes of CD4+/CD8+ T cell ratio in immunosuppressive mice

Hydrocortisone decreased the ratio of peripheral blood T-lymphocyte subsets ($CD4^+/CD8^+$) significantly. The percentages of $CD4^+$ T-cells in Chinese herbal groups were significantly higher than those of NS+Hy (P<0.05). However, there was no significant difference between the percentages of $CD8^+$ T-cells in Chinese herbal group and NS+Hy group (P>0.05). In addition, the ratios of $CD4^+/CD8^+$ in the low, middle and high dose groups were significantly higher than those in Hy+NS (P<0.01). However, no significantly difference was observed between the herbal and NS groups (P>0.05) (See Table 4).

Table 4: Effect of Chinese herbal medicine on CD4+/CD8+ T cell ratio in immunosuppressive mice

Groups	Dosage(mL/mouse)	Sample (n)	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
NS	0.4	10	34.79±9.25	15.68±4.47	2.29±0.56
NS+Hy	0.4	10	33.18±7.48	18.24±3.78	1.81±0.05a
L+Hy	0.2	10	41.03±7.38*	15.81±1.87	2.62±0.55**
M+Hy	0.4	10	38.55±6.46*	16.05±3.12	2.43±0.28**
H+Hy	0.6	10	41.60±5.56*	16.98±2.96	2.47±0.22**

Note: Compared with NS+HY control group,**(P<0.01);* (P<0.05); compared with NS control group, A (P<0.01), a (P<0.05)

Discussion

It is an important key problem to search for highly effective immunomodulators without side effects in the field of medical and biological research (Zheng and Xu,2004; Phillips et al ,2006; Dannull et al ,2005). In this paper, the immune modulating effects of the Chinese herbal formula consisting of *Astragalus membranaceus*, *Epimedium brevicornum*, *Paeoniae Alba Radix* and *Radix Ophiopogonis* were studied. The herbal formula contains active components of polysaccharide, saponin, flavonoids and other active ingredients which have been shown to activate immune system and immunocytes. The Chinese herbal recipe was formulated on the base of modern theories of Veterinary Medicine and Science compatibility, by administrating different doses of the recipes to observe the effect of immunologic enhancement in ICR mice.

Under synergies of Macrophage and T-lymphocytes, activated B-cells proliferate and differentiate

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into plasma cells when exposed to antigen stimulation synthesis. These plasma cells excrete into blood or tissue fluid, therefore activating the humoral immune reaction. In the humoral immune effect stage, antigen recognition is mediated by binding the secretion of antibodies and antigens (Xue, 1995; Yang, 2002). Hemolytic plaque test (PFC) is the way of checking and counting IgG, IgM antibody forming cells (AFC). Splenocyte suspension, SRBC and serum from Guinea pigs were reacted in liquid phase medium, QHS（quantify hemolysis spectrophotometric analysis) was a commonly used method. Immune splenocytes, sheep red blood cells (SRBC) and complement were reacted in a liquid medium to observe the release of hemoglobin content and then extrapolated the amount of antibody secretion of AFC. This paper demonstrated that the middle and high-dose groups could significantly countermine the lowering of AFC function by Hydrocortisone (Hy). It was shown that the humoral immunity function was related to the secretion of AFC in mice.

SRBC antibody haemolysin was produced and released to peripheral blood by injecting SRBC into mice. Then the content of haemolysin was observed by measuring the value of HC50. From the above experimental data, we knew low, middle and high dose groups could significantly enhance the level of serum hemolysin in immunosuppressive mice. The results reported by other researchers (Cao et al., 2003; Luo et al., 2005; Tang et al, 2005) showed that composition of *Astragalus* and *Epimedium* could enhance the level of antibody compared with that in control group ($P>0.05$). In addition, Epimedium polysaccharide and icariin could countermine mice spleen diminution and the reduction of antibody formation. Especially, the effect was better than when they were combined. The Chinese herbal medicine prescription was made up of Epimedium, Astragalus and so on. This demonstrated that the prescription had the function of enhancing humoral immunity.

T-lymphocytes could be classified into two subgroups, $CD4^+$ T-cells (Th) and $CD8^+$ T-cells (Ts/Tc), in accordance with distinct cluster of differentiation (CD) on the cell membrane. They could be further classified into Th1 and Th2, both of which play an important role in regulating the immune response. Ts could restrain B cells to produce immunoglobulin, and simultaneously restrain the reaction of T-lymphocyte to mixed lymphocytes. However, Th cells could maintain a normal immunological balance by assisting B cells to produce immunoglobulin. The dynamic balance of $CD4^+$ and $CD8^+$ is a significant component that reflected the state of cell-mediated immunity. The number of cells involved in cell-mediated immunity could be determined by measuring the numbers of T-lymphocytes, the $CD4^+$ and $CD8^+$ subsets and the ratio of $CD4^+/CD8^+$. Other studies have indicated that the emergence and development of gallbladder cancer was closely related to T-cell function. Especially, the ratio of $CD4^+/CD8^+$ could indicate the state and capacity of cell-mediated immunity. According to the above results we knew that the Chinese herbal medicine could enhance the ratio of $CD4^+/CD8^+$ and the level of cell immunity. This was in accordance with the report by Hong et al. (2005). In addition, some literatures (Song et al., 2005; Mao et al., 2006; Yang et al., 2002) reported that water-solubility of Astragalus and astragalus polysaccharides could enhance the level of lymphocyte, especially enhance the ratio of $L3T4^+/Lyt2^+$ immune hypofunction mice by Hydrocortisone (Hy). Other findings also confirmed this view point (Sheng and Hong, 2007; Lodon and Lanier,2005; Cormary et al., 2004).

From the above results, we come to the conclusions that Chinese herbal recipes may be a good immunoenhancer, especially in immune suppression mice. It can promote T-lymphocytes proliferation, cause metabolism activation, enhance the function of cellular and humoral immunity, and defend immunization against traumatise and immunologic injury recovery.

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