Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7):1723-1726

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Influence of Sarcodon imbricatus polysaccharide on immune function in immunosuppressive mouse

Silu Wang[1], Kaiyu Wang*[2], Defang Chen[3] and Ling Zhao[2]

[1]*Department of Animal Science, Xichang College, Xi'chang, PR China*
[2]*Department of Animal Medicine, Sichuan Agricultural University, Ya'an, PR China*
[3]*Department of Aquaculture, College of Animal Science & Technology, Sichuan Agricultural University, Ya'an, PR China*

ABSTRACT

The objectives of this study were to investigate the immune enhancement activity of the Sarcodon imbricatus polysaccharide (SIP). In this study, water extraction method was employed to isolate polysaccharides from the Sarcodon imbricatus. The immunoloregulation effects of SIP were evaluated with models of cyclophosphamide-induced mouse immunosuppression. The immunoloregulation activities of SIP were investigated using T lymphocyte subpopulation assays. The ability of these polysaccharides to stimulate mouse cytokine and splenocyte proliferation were measured using ELISA test and MTT reagent. The results revealed that SIP could significantly increase CD3+% and decrease CD4+/CD8+ with dose-dependent in vivo. In serum, IL-2 and IFN-γ content were raised after the immunosuppressive mouse were treated by SIP, meanwhile the serum IL-4 content was decreased. SIP also significantly promoted splenocyte proliferation. The immunoenhancement activities of SIP was better than astragalus polysaccharides (APS) with same dose. Outcomes of this research strongly indicate that SIP display significant immunoenhancement activities to immunosuppressive mouse, and have therapeutic potential to be used for the treatment of immunosuppressive disease.

Key words: Sarcodon imbricatus, polysaccharide, T lymphocyte subpopulation, cytokine, lymphocyte proliferation

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INTRODUCTION

During the past decade, researches on polysaccharides have gained application for medicine due to their immunoloregulation value and minimum side effects[1-4]. In China, Sarcodon imbricatus is believed to possess medicinal properties, such as strengthening body, improving thought processes and preventing aging. To investigate the biologic activity of Sarcodon imbricatus as drugs and nutraceuticals, previous research showed that Sarcodon imbricatus polysaccharides（SIP）could remarkably inhibit the growth of human liver cancer cell line Hep G2 and human ovarian cancer cell line HO-8910 in a dose-dependent manner in vitro[5]. The total phenols and flavonoids in the extracts of Sarcodon imbricatus inhibited the growth of Gram positive bacteria (Bacillus cereus, B. subtilis,), while Escherichia coli (Gram negative bacteria) was resistant[6]. Our previous works showed that SIP had remarkable immune enhancement activity to normal mouse[7]. As a logical extension of the above discussion, this study is to investigate the immunological activity of SIP to immunosuppressive mouse.

EXPERIMENTAL SECTION

SIP AND ASTRAGALUS POLYSACCHARIDES (APS)

Sarcodon imbricatus was decocted with distilled water and the ultrasonic extraction technology were used. The SIP

was extracted by 75% ethanol from decoction[8]. The content of polysaccharide in SIP was 91.3% measured by the phenol–sulfuric acid method. For test in vivo, SIP was diluted to three working concentrations (5, 10, 20mg/ml), and the peritoneal injection dosages of SIP were 0.2ml. The extracted methods of APS was same as SIP, the polysaccharide content of APS was 93.7% .

ANIMALS AND TREATMENT

Female KM mice (20±2g) were purchased from the Experimental Animal Center of Sichuan University (Certificate Number: HX20131107, Chendu, China). The mice were divided into 6 groups, they were blank group (0.2ml 0.9% NaCl, ip), Cyclophosphamide (Cy) group (0.2ml 0.9% NaCl, ip)，positive control group (0.2ml 20 mg/ml APS, ip) and three SIP groups (0.2 ml 5,10 and 20 mg/ml SIP, ip). After all groups were treated as above for 7 days, at fourth day the mice of each group except blank control group were peritoneal injected Cy 2.5 mg/mouse for 3 days.

T LYMPHOCYTE SUBPOPULATION ASSAY

Briefly, 100 μl anticoagulated blood of each mouse was respectively stained with 5 μl hamster anti-mouse CD3-FITC, 5 μl rat anti-mouse CD4-PE and 5 μl rat anti-mouse CD8-PerCP for 30min in dark. Then 1ml hemolysin was added, 10 min later centrifuged at 2000 r/min for 5min[9], peripheral lymphocytes were collected and washed with 2ml PBS two times, then suspended lymphocytes in 500 μl PBS and determined by flow cytometry analyses (BD FACS Calibur TM , USA).

SERUM IL-2, IFN-γ AND IL-4 CONTENT ASSAY

The serum IL-2, IFN-γ and IL-4 were assessed using commercially available kits (BOSTER, Wuhan, China) according to the kits' manual.

SPLENOCYTE PROLIFERATION ASSAY

The spleens were respectively removed from each grroup, minced into small pieces, and passed through a tissue sieve (200 mesh per 2.5 cm) with PBS. The splenocyte suspension was centrifuged at 1500 r/min for 3 min, the supernatant was discarded, then treated the precipitate with 1ml hemolysin for 5min, then the suspension was centrifuged at 1500 r/min for 3 min. Splenocytes were collected and washed with RPMI 1640 media twice, and 95% of them were alive. The splenocytes in RPMI-1640 medium supplemented with 10% fetal calf serum, used at the concentration of 1×10^6 cells/ml. The splenocytes suspension from different group were divided into 3 parts, respectively treated with 25 mg/ml ConA, 10 mg/ml LPS and nothing. The cells were then incubated for 44 h at 37°C with 5% CO_2[10-12], and further 4 h with 20 μl 2 mg/ml MTT per well. Then 150 μl of dimethyl sulfoxide was added to the culture and vibrated for at least 10 min to fully dissolve the colored material. The absorbance at 570 nm of each sample was measured in an ELISA reader (Bio-Rad, USA).

STATISTICAL ANALYSIS

The results were mentioned as mean ± SD and proceeded to statistical analyse using SPSS 17.0. Duncan's multiple range test was used to evaluated the difference. The level of significance for all analysis was taken as $p<0.05$ or $p<0.01$.

RESULTS AND DISCUSSION

Assessment of T Lymphocytes Subgroups in immunosuppressive mouse

Compared with the blank control group, Cy reduced the total count of splenic lymphocytes, and significantly increased CD3+ and CD4+ positive rate of T lymphocytes, while reducing CD8+ ratio. Thus, CD4+/CD8+ ratio of all groups significantly increased. APS and SIP also significantly increased the ratio of CD3+ and CD4+. With the increasing SIP dose, the ratio of CD3+ and CD8+ ratio also showed an increasing tendency, and CD4+ ratio was reduced. (Table 1)

Compared with the Cy group, SIP at a high and medium dose extremely increased the CD3+ ratio. However, the difference between APS group and low dose SIP group was not prominent. APS and high dose SIP groups showed a decreasing tendency with CD4+. Between them, SIP at a high dose presented the optimal performance, and the two groups did not show significant difference. APS and different doses of SIP could lift CD8+ in immunosuppresive mouse with different degrees. SIP at a high dose promoted the CD8+ ratio to 73.56%, which was greatly higher than 4.4% of the positive group. SIP at a high or medium dose could extremely reduce CD4+/CD8+ ($P<0.05$). The positive group showed a tendency to reduce CD4+/CD8+ ratio, but the tendency was not obvious. (Table 1)

In the experiment, it was found that the total count of lymphocytes in mouse with Cy injected was reduced, while

CD3+ ratio significantly increased. This indicated that Cy may perform more cytotoxicity to B lymphocytes and monocytes. Meanwhile, SIP significantly increased CD3+% and CD8+% of immunosuppressive mouse, while also enlarging the total count of lymphocytes. It indicated that SIP promoted the proliferation of T cells as well as B cells and monocytes. In addition, CD4+/CD8+ of mouse with immunosuppression was also greatly higher than the normal value. SIP at a high dose could elevate CD8+% while reducing CD4+%, so as to significantly decrease the CD4+/CD8+ ratio to the normal value. In the study SIP could improve Cy induced immune dysfunction of cells with better performance than APS.

Table 1. Effect of SIP on lymphocyte subpopulation in immunosuppressive mouse（$\bar{x}$ ± s， n=10）

Group	CD3+（%）	CD4+（%）	CD8+（%）	CD4+/CD8+
Blank control	73.03±4.72^{a}	36.43±3.51^{a}	32.37±2.99^{a}	1.13±0.17^{a}
Cy	90.12±4.51^{b}	74.05±6.03^{b}	16.45±1.50^{b}	4.50±0.68^{b}
Cy+APS	93.17±4.06^{b}	73.32±6.11^{b}	17.18±1.67^{b}	4.27±0.59^{b}
Cy+high dose SIP	97.91±2.02^{c}	69.72±5.32^{b}	28.55±4.58^{c}	2.44±0.52^{c}
Cy+medium dose SIP	96.21±2.07^{c}	75.51±6.13^{b}	19.17±1.65^{b}	3.94±0.48^{B}
Cy+low dose SIP	93.12±4.10^{b}	76.28±3.05^{b}	18.21±2.37^{b}	4.18±0.78^{b}

Note: Same letters with different size in the same array means difference between the treatments (P<0.05), different letters in the same array means significant difference between the treatments (P<0.01).The same as below.

Assessment of cytokines in peripheral blood of immunosuppressive mouse

Compared with the blank control group, IL-2, IL-4 and IFN-γ content in the serum of mouse in each handled group all dropped signficantly. Compared with the Cy group, APS and different doses of SIP could significantly increase IL-2(P<0.01) and IFN-γ(P<0.05). Difference of cytokines between high dose SIP group and the positive group was extremely significant(P<0.01). The research outcomes showed that SIP at a high dose increased the IL-2 and IFN-γ content by 202.08 % and 87.60 % for immunosuppressive mouse. The difference of IFN-γ between high dose group and the blank control group was not sinificant. The effects on IL-4 showed that APS and SIP might generate synergistic effects with cyclophosphamide, which further reduced IL-4 content of mouse. The reduction was inversely proportional to SIP dose. Compared with the Cy group, IL-4 content of the APS group dropped by 33.88 %. Meanwhile, the low dose SIP group dropped 60.13 %. (Table 2)

The research outcomes demonstrated that SIP at a high dose could prominently promote the IL-2 and IFN-γ concentrations in peripheral blood of mouse with immunosuppression. As IL-2 can promote the proliferation of lymphocytes, and induce killer cells to generate IFN-γ, TNF-α and other cytokines, and activate macrophages. This indicated that SIP could positively regulate the immunoreactions. Meanwhile, IL-4 is the factor to promote the proliferation of B cells and negatively regulates the immunoreactions. SIP at a low dose performed significant depression on IL-4.

Table 2. Effects of SIP on cytokine in immunosuppressive mouse（$\bar{x}$ ± s， n=10）

Group	IL-2（pg/ml）	IL-4（pg/ml）	IFN-γ（pg/ml）
Blank control	16.27±2.21^{a}	27.38 ±1.54^{a}	326.01±46.27^{a}
Cy	4.33± 0.78^{b}	19.44±2.57^{b}	163.07±36.71^{b}
Cy+APS	8.54±1.66^{c}	14.52±1.71^{c}	226.81±35.16^{c}
Cy+high dose SIP	13.08±2.11^{d}	16.17±1.57^{d}	305.92±33.57^{a}
Cy+medium dose SIP	9.36±1.47^{c}	14.50±2.07^{c}	218.03±34.82^{c}
Cy+low dose SIP	8.33±1.05^{c}	12.14±1.82^{e}	203.62±46.84^{cB}

Assessment of lymphocyte proliferation in immunosuppressive mouse

The proliferation of T and B lymphocytes in each handled group with or without ConA or LPS was less efficient than the blank control group. With the synergy of ConA or LPS, APS and SIP at a high and medium dose could significant promote the proliferation of T or B splenic lymphocytes of immunosuppressive mouse . Meanwhile, without ConA or LPS, APS and different doses of SIP could significantly promote the proliferation of T or B lymphocytes of immunosuppressive mouse(P<0.01), indicating that the two polysaccharides could act similarly to mitogen, they could directly promote the proliferation of lymphocytes on a SIP dose-dependent basis. The results showed that SIP at a high and medium dose could promote the proliferation of lymphocytes in a similar to enhanced way than APS (P<0.01). (Table 3)

Table 3. Effects of SIP on splenic lymphocyte proliferation in immunosuppressvie mouse（$\bar{x} \pm s$，n=3）A_{570}

Group	+10 μg/ml LPS	+2.5 μg/ml ConA	—
Blank control	0.465±0.022[a]	0.416±0.031[a]	0.322±0.014[a]
Cy	0.203±0.020[b]	0.260±0.012[b]	0.155±0.011[b]
Cy+APS	0.332±0.035[c]	0.347±0.035[c]	0.214±0.018[c]
Cy+high dose SIP	0.416±0.031[d]	0.401±0.024[d]	0.279±0.014[d]
Cy+medium dose SIP	0.339±0.027[c]	0.366±0.023[d]	0.245±0.011[d]
Cy+low dose SIP	0.281±0.028[e]	0.268±0.018[b]	0.173±0.009[e]

Cyclophosphamide (Cy) is a common cytotoxicity chemotherapeutics drug, and an immunosuppressor. It can prominently depress the immune function of mouse cells and establish a long-term immunosuppressive model. Our research injected 2.5mg/mouse Cy intraperitoneally in mice for 3 consecutive days to accomplish the modeling. Thus, proliferation and differentiation of splenic lymphocytesm, and the IL-2, Il-4 and IFN-γ concentrations of cytokine in peripheral blood were reduced significantly. These indicated that the immunologic function of mouse dropped significantly. The proliferation ability of splenic lymphocytes is one of the important indexes to assess the cellular immune function. As spleen contains T and B lymphocytes, ConA can promote the fragmentation of T lymphocytes and LPS can promote the fragmentation of B lymphocytes. Our research showed that SIP at a high dose can individually and prominently promote the proliferation of splenic lymphocytes of immunosuppressive mouse. SIP can also form the synergistic effect with ConA or LPS, indicating that SIP could promote the proliferation of activated lymphocytes.

CONCLUSION

From the results of immunoloregulation activity, it can be concluded that different doses of SIP might conduct dual-direction regulations to cells and humoral immunity. SIP could greatly improve cellular immune function for mouse with immunosuppression. Thus, it is expected that SIP can be a new safe and efficient immunomodulator, but its mechanism should be further studied.

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