ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12): 1121 - 1124
http://www.chinaphar.com

Chemo- and radio-protective effects of polysaccharide of *Spirulina platensis* on hemopoietic system of mice and dogs

ZHANG Hong-Quan[1], LIN An-Ping, SUN Yun, DENG Yang-Mei
(*The Medical and Pharmaceutical Academe of Yangzhou University, Yangzhou 225001, China*)

KEY WORDS *Spirulina platensis*; polysaccharides; bone marrow; gamma rays; radiation-protective agents; hemoglobins; erythrocyte count; leukocyte count

ABSTRACT

AIM: To observe polysaccharide of *Spirulina platensis* (P*Sp*) on the hematopoietic system of mouse and dogs which were damaged by injection of cyclophosphamide (CTX) and ^{60}Co-γ irradiation. **METHODS**: CTX and ^{60}Co-γ ray were used to induce bone marrow damage, and the experimental animals were ig with different dose of P*Sp in vivo*, after 12-d and 21-d administration, the whole blood cells and nucleated cells in bone marrow were measured, and the DNA in bone marrow were inspected by UV-spectrophotometer. **RESULTS**: CTX and ^{60}Co-γ irradiation induced hemopoietic system damage in mice and dogs, respectively. P*Sp* 30, 60 mg/kg increased the level of the white cells in blood and nucleated cells and DNA in bone marrow in mice but had no effects on red cells and hemoglobins. P*Sp* 12 mg/kg increased the level of red cells, white cells, and hemoglobins in blood and nucleated cells in bone marrow in dogs ($P < 0.01$), and the effects of P*Sp* 60 mg/kg were better than that of berbamine hydrochloride 60 mg/kg. **CONCLUSION**: P*Sp* has chemo-protective and radio-protective capability, and may be a potential adjunct to cancer therapy.

INTRODUCTION

Polysaccharide of *Spirulina platensis* (P*Sp*) is an acidic polysaccharide attracted from *Spirulina*, containing rhamnose, fucose, xylose, mannose, glucose, and galactose. Its molecular weight (M_r) is between 3.4×10^3 and 2.9×10^6. There were many researches reported that it had anti-oxidant and anti-fatigue effects[1], and increased the immune capability in body and promoted DNA synthesis. In plant it can prevent cells from irradiation damage, and enhance DNA repair and synthesis capability[2].

Chemotherapy and radiotherapy, common methods in tumor therapy, not only kill tumor cells, but also make many adverse effects primarily because of damage of the hematopoietic system[3]. As a natural agent, P*Sp* has little toxicity to human, and its usage in tumor therapy attracts much attention. *In vivo* and *in vitro* research finds indicated that it suppressd the growth of tumor cells, promoted NK cells activity, and induced lymphocyte in spleen to produce TNF-α[4]. It is also found that P*Sp* can increase the rate of survive of mice and promote hematopoietic stem cells and progenitor cells to differentiate after lethal ^{60}Co-γ ray irradiation[5]. Based on the characteristics of low toxicity and anti-tumor activities, we want to study whether P*Sp* has some effect on hematopoietic system after chemical and radical damage.

MATERIALS AND METHODS

Materials P*Sp* were supplied by China Pharmaceutical University, polysaccharide acounted for 63.5 % (970912). Berbamine hydrochloride (BH) was purchased from the Wuxi № Six Pharmaceutical Factory (№ 960112). Cyclophosphamide (CTX) was produced by Shanghai № 12 Pharmaceutical Factory (№ 960501); Cesium-137 radiation of ^{60}Co-γ (Subei Renmin Hospital, 100 Gy/min).

Animals ICR mouse of either sex (Grade Ⅱ, Certificate № 93104) were purchased from Central Experimental Animal Facility of Yangzhou University. The animals, 2-month-old, 18 – 22 g, were housed 10 per cage. The dogs (№ 9100013) weighed between 5.8 and 7.2 kg. All the animals were allowed free access to food and water during experiment.

Effect of P*Sp* on hemopoietic system of mice

[1] Correspondence to Prof ZHANG Hong-Quan.
Phn 86-514-797-8877. Fax 86-514-734-1733.
Received 2001-02-16 Accepted 2001-06-14

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

induced by CTX Sixty ICR mice were divided into 6 groups. The control group were ig administered distilled water 15 mL·kg·d^{-1} and the positive control group were given ig BH 60 mg·kg·d^{-1} for 12 d. The other three P*Sp*-treated groups were given ig P*Sp* 15, 30, and 60 mg·kg·d^{-1} for 12 d. After consecutive administration for 5 d, except for the control group, all the mice were injected CTX ip 100 mg·kg·d^{-1} for 3 d.

On the 12th day, the white and red blood cell (WBC and RBC) number were counted and hemoglobin were measured, respectively.

For DNA determination, on d 12, the mice were sacrificed and the femurs of both sides were obtained. The cavity of right femur were washed with 10 mL $CaCl_2$ using a sterile syringe and a 26-gauge needle. The solution was placed into an icebox at 4 ℃ for 30 min, then centrifuged at 2500 × *g* for 15 min. After the supernatant was casted off, $HClO_4$ 0.2 mol/L 5 mL was added in the precipitant and mixed thoroughly, then heated at 90 ℃ for 15 min. The liquid were passed through stainless steel meshnets after cooling. The optical density of the liquid were measured under UV-spectrophotometer at 260 nm. (1 unit of OD_{260} = dsDNA 50 mg/L. DNA in per femur = *OD* value × 50 × 5 μg.

The cavity of the left femur was washed with 10 mL 3 % acetic acid to get the bone marrow cells. Cell counts were obtained using a hemocytometer.

Effect of P*Sp* on hemopoietic system in dogs induced by ^{60}Co-γ irradiation in dogs Normal dogs (n = 20) were divided into 5 groups and fed routinely for 1 week before experiment. The normal control group and model group (^{60}Co-γ irradiation group) were fed with pig intestines which has no drug. The P*Sp*-treated groups were fed with P*Sp*, which were mixed with meat, at the dose of 3 and 12 mg·kg·d^{-1} for 26 d, respectively. The positive control group were fed with BH 12 mg·kg·d^{-1} for 26 d. On d 5, the dogs were irradiated by ^{60}Co-γ ray at 100 Gy/min. White and red blood cells, bone marrow cells, and hemoglobin were measured on d 7, 14, and 21, respectively.

Statistical analysis Data were expressed as $x \pm s$ and analyzed by paired *t*-test. $P < 0.05$ was considered significant.

RESULTS

Effect of P*Sp* on the decrease of peripheral blood cells induced by CTX CTX decreased peripheral blood cells including white blood cells, red blood cells, and hemoglobin. P*Sp* 30 and 60 mg·kg·d^{-1} for 12 d increased the white blood cells to 15 % – 60 %, and the effect of P*Sp* 60 mg·kg·d^{-1} was better than BH, but they had no effect on the red blood cells and hemoglobin (Tab 1).

Tab 1. Effect of P*Sp* on the decrease of peripheral blood cells induced by CTX. $n = 10$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control. $^{f}P < 0.01$ *vs* CTX 100 mg·kg·d^{-1}.

Group	10^{-9} × WBC/ L^{-1}	10^{-12} × RBC/ L^{-1}	HB/ g·L^{-1}
Control	11.5 ± 2.6	5.3 ± 0.4	138 ± 12
CTX (100 mg/kg × 3 d)	3.37 ± 0.29^{c}	4.13 ± 0.18	130 ± 3^{c}
CTX + BH (60 mg/kg)	4.5 ± 0.9^{fc}	4.4 ± 0.4^{b}	137 ± 10^{c}
CTX + P*Sp* (15 mg/kg)	3.9 ± 0.4^{fc}	4.16 ± 0.17^{c}	126 ± 5^{c}
CTX + P*Sp* (30 mg/kg)	4.5 ± 0.7^{fc}	4.3 ± 0.3^{c}	132 ± 12^{c}
CTX + P*Sp* (60 mg/kg)	5.5 ± 1.3^{fc}	4.36 ± 0.22^{c}	132 ± 9^{c}

Effect of P*Sp* on the decrease of DNA and nucleated cells in bone marrow induced by CTX CTX decreased the DNA and nucleated cells in bone marrow, which indicated a distinct suppression on bone marrow. P*Sp* increased levels of the DNA and nucleated cells in the suppressive state. The effect of P*Sp* 60 mg·kg·d^{-1} is better than BH (Tab 2).

Tab 2. Effect of P*Sp* on the decrease of DNA in bone marrow induced by CTX. $n = 5$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control group. $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* CTX group.

Group	Content of DNA/ μg per femur	10^{-6} × Nucleated cells per femur
Control	270 ± 48	10.0 ± 1.4
CTX (100 mg/kg)	96 ± 36^{c}	1.16 ± 0.16^{c}
CTX + BH (60 mg/kg)	209 ± 72^{e}	7.0 ± 1.8^{ef}
CTX + P*Sp* (15 mg/kg)	198 ± 40^{bf}	2.7 ± 0.6^{ef}
CTX + P*Sp* (30 mg/kg)	155 ± 24^{cf}	4.9 ± 0.4^{ef}
CTX + P*Sp* (60 mg/kg)	223 ± 87^{e}	7.4 ± 1.7^{ef}

Effect of P*Sp* on decrease of peripheral blood cells and nucleated cells in bone marrow induced by ^{60}Co-γ irradiation in dogs ^{60}Co-γ irradiation reduced white blood cells, red blood cells, and hemoglobin in peripheral blood to 62.5 %, 26.4 %, and 18.9 % respectively. P*Sp* 12 mg/kg increased the levels of white and red blood cells and hemoglobin (Tab 3, 4).

Tab 3. Effect of P*Sp* on the decrease of white blood cells caused by ^{60}Co-γ irradiation. $n = 4$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control group. $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* model group.

Group	$10^{-9} \times$ WBC/L^{-1} d 7	d 14	d 21
Normal control	12.0 ± 2.5	10.8 ± 2.3	11.6 ± 2.5
Model(^{60}Co irradiation)	4.5 ± 0.4^{c}	4.9 ± 1.2^{c}	7.1 ± 1.5^{b}
^{60}Co + BH(12 mg/kg)	6.0 ± 0.9^{ce}	7.0 ± 1.3^{b}	8.0 ± 0.4^{b}
^{60}Co + P*Sp*(3 mg/kg)	4.7 ± 0.5^{c}	5.30 ± 0.18^{c}	7.2 ± 2.2^{b}
^{60}Co + P*Sp*(12 mg/kg)	6.3 ± 0.9^{ce}	7.1 ± 0.8^{c}	8.6 ± 0.9^{b}

After 7 d ^{60}Co-γ irradiation, the grannulocyte system and erythroid system were greatly suppressed. P*Sp* decreased the injury coursed by ^{60}Co-γ irradiation, and the effect of P*Sp* 12 mg/kg is better than that in BH 12 mg/kg group (Tab 5).

DISCUSSION

Chemotherapy and radiotherapy are the most commonly used methods in cancer treatment, but they inevitably led to a decrease in bone marrow function which was reflected by a substantial decrease in mature blood elements[6]. In this experiment the DNA and nucleated cells in bone marrow and the level of peripheral blood cells and hemoglobin were all decreased markedly after CTX or ^{60}Co-γ ray treatment indicating that chemical or radiant injury model was established, respectively.

Our results showed that P*Sp* 30 and 60 mg/kg in mice as well as 3 and 12 mg/kg in dog could increase the level of white cells and hemoglobin in blood and nucleated cells in granulocyte system and erythroid system. Liou *et al* reported that it could prevent the bone marrow cells in mouse from injury induced by irradiation[7]. Pang *et al*[8] studied the radioprotective effect of an extract of *Spirulina platensis* using the micronucleus test in polychromatic erythrocytes of bone marrow of mice. In this system the extract caused a marked reduction of the micronucleus frequencies induced by gamma-radiation. The prior research in our laboratory indicated that P*Sp* protected DNA of 2BS cells from damage induced by UV irradiation. All above findings were consistant with the present results. Xu *et al* found it could increase spleen weight and the number of lymphocyte and promote spleen lymphocyte transformation in mice exposed to gamma-ray[9]. Also as a natural agent, glycosides of cistanche (GCS) has similar effect as P*Sp* on hematopoietic system of ^{60}Co-γ ray irradiated mice, and the mechanism of this effect has relevance to its anti-oxidant capability[10]. The radiation-protective effects of P*Sp* may be related with its anti-oxidant and anti-fatigue capability[11] and immuno-proliferative effects.

In summary, P*Sp* had chemo-protective and radio-protective capability and was a potential agent for the restoration of hematopoesis in patients after radiotherapy and chemotherapy.

Tab 4. Effect of P*Sp* on the decrease of red blood cells and hemoglobin in peripheral blood cells caused by ^{60}Co-γ. $n = 4$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control group. $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* model group.

Group	d 7		d 14		d 21	
	$10^{-12} \times$ RBC/L^{-1}	HB/g·L^{-1}	$10^{-12} \times$ RBC/L^{-1}	HB/g·L^{-1}	$10^{-12} \times$ RBC/L^{-1}	HB/g·L^{-1}
Normal control	6.6 ± 0.6	152 ± 16	6.5 ± 1.0	149 ± 10	6.4 ± 0.8	143 ± 17
Model (^{60}Co irradiation)	4.9 ± 0.6^{c}	123 ± 13^{b}	5.9 ± 0.6	137 ± 13	6.2 ± 0.8	144 ± 13
^{60}Co + BH(12 mg/kg)	5.5 ± 0.7	138 ± 14	6.2 ± 0.4	151 ± 15	6.4 ± 0.7	146 ± 18
^{60}Co + P*Sp*(3 mg/kg)	5.1 ± 0.8^{e}	127 ± 6b	5.6 ± 0.6	141 ± 5	5.6 ± 0.7	144 ± 8
^{60}Co + P*Sp*(12 mg/kg)	6.1 ± 0.3^{f}	149 ± 14^{e}	6.8 ± 0.3^{e}	154 ± 14	6.4 ± 0.5	158 ± 17

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Tab 5. Effect of PSp on the suppression of hematopoietic system caused by ^{60}Co-γ. $n = 4$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{B}P < 0.01$ *vs* control group. $^{e}P < 0.05$, $^{E}P < 0.01$ *vs* model group.

Group	Nucleated cells d 7	d 14	d 21
	In granulocyte system/%		
Normal control	49 ± 5	46 ± 6	48 ± 4
Model(^{60}Co irradiation)	2.2 ± 1.7	10 ± 4^{c}	46 ± 5^{b}
^{60}Co + BH(12 mg/kg)	5.5 ± 3.0^{ce}	20.4 ± 2.9^{bf}	47 ± 8
^{60}Co + PSp(3 mg/kg)	2.9 ± 1.1^{c}	16.3 ± 1.7^{ce}	46 ± 3
^{60}Co + PSp(12 mg/kg)	7.7 ± 1.1^{cf}	22.3 ± 2.9^{cf}	46 ± 6
	In erythroid system/%		
Normal control	33 ± 4	29.3 ± 2.4	31 ± 3
Model(^{60}Co irradiation)	14 ± 6^{b}	19 ± 6	27 ± 6
^{60}Co + BH(12 mg/kg)	18.0 ± 2.9^{c}	21.0 ± 2.1	29 ± 5
^{60}Co + PSp(3 mg/kg)	14 ± 4^{c}	18.8 ± 2.9^{c}	29 ± 6
^{60}Co + PSp(12 mg/kg)	22 ± 4^{c}	24 ± 4	31 ± 4

REFERENCES

1 Zho SY. Experimental research on the anti-oxidation and anti-fatigue effect of the polysaccharide from *Spirulina platensis* cultivated in Yunnan. Chin J Biochem Pharm 1995; 16: 255 – 8.
2 Pang QS, Guo BJ, Ruan JH. Effect of polysaccharide from *Spirulina platensis* on the activity of endonuclease and on DNA repair-synthesis capability. Acta Genet Sin 1988; 15: 374 – 81.
3 Crearen PJ, Mihchi E. The clinical toxicity of anti-cancer drugs and its prediction. Semin Oncol 1977; 41: 47 – 9.
4 Qu XJ, Cui SX, Xie SY. Antitumor studies on the polysaccharides *Spirulina platensis in vivo*. Chin J Mar Drugs 2000; 19: 10 – 4.
5 Wang YL, Chen WH, Xie YX. Study on the pharmacologic functions of the polysaccharide of *Spirulina* maxima. Biotech Bull 1999; 15: 26 – 9.
6 Hoagland HE. Hematological complication of cancer chemotherapy. Semin Oncol 1982; 9: 95 – 7.
7 Liou LS, Guo BJ, Ruan JH. The research on the inhibitory activity of polysaccharide from *Spirulina platensis* on transplant murine cells. Ocean Sci 1991; 9: 33 – 5.
8 Pang QS, Guo BJ, Kolman A. Radioprotective effect of extract from *Spirulina platensis* in mouse bone marrow cells studied by using the micronucleus test. Toxicol Lett 1989; 48: 165 – 9.
9 Xu H, Yu ZG, Shu HQ. Effect of polysaccharides from *Spirulina platensis* on immunological function of mice exposed to gamma-ray. Radiat Res Radiat Tech J 1997; 15: 186 – 8.
10 Jiang XY, Wang XW. Protective effects of glycosides of cistanche on hematopoietic system of ^{60}Co-ray irradiated mice. Chin Pharmacol Bull 2000; 16: 332 – 5.

螺旋藻多糖对小鼠和犬造血系统的化学和放射防护作用

张洪泉[1]，林安平，孙　云，邓杨梅
（扬州大学医药研究所，扬州 225001，中国）

关键词　螺旋藻；多糖类；骨髓；γ 射线；辐射防护剂；血红蛋白类；红细胞计数；白细胞计数

目的：研究螺旋藻多糖（PSp）对环磷酰胺和^{60}Co-γ 射线所致小白鼠和犬造血系统抑制的影响．**方法**：腹腔注射环磷酰胺以及用^{60}Co-γ 射线照射分别诱发小鼠和犬的骨髓损伤．全血细胞计数和骨髓有核细胞计数．用紫外分光光度计检测骨髓 DNA 的含量．**结果**：环磷酰胺和^{60}Co-γ 射线分别造成小鼠和犬骨髓造血系统抑制性损伤．PSp 30，60 mg/kg 能升高小鼠全血白细胞数和骨髓有核细胞数以及 DNA 含量；PSp 12 mg/kg 能使犬骨髓有核细胞数，以及外周血红细胞、白细胞及血红蛋白水平得以回升（$P < 0.01$），其疗效优于盐酸小壁胺．**结论**：PSp 对造血系统有化学保护和放射保护作用．

（责任编辑　朱倩蓉）