

Article

Phycocyanin / Polysaccharide of Spirulina Platensis for Antitumor Immune Activity

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Abstract: Phycocyanin (PC) and polysaccharides from spirulina plantensis (PSP) are immune active substances with great anti-tumor potential. The antitumor immune activities of PC and PSP were studied in this paper. The antitumor immune activities of alginate and polysaccharide were detected by ELISA, MTT assay and quantitative hemolysis spectrophotometry at the levels of immune organs, immune cells and immune molecules. The results showed that the tumor diameter and tumor mass of mice treated with PC or PSP were smaller than those in the control group, the activity of T cells and B cells was also significantly enhanced, and the number of humoral antibodies was significantly increased. The effect of PS on tumor cell growth and immunity was more obvious than that of PSP.

Keywords: Phycocyanin; Polysaccharides; Immunoactivity; Anti-tumor

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1. Introduction

Spirulina is a kind of lower plant, which is a photosynthetic autotrophic organism. It contains a large amount of proteins, polysaccharides and a variety of bioactive substances. These substances are widely valued for their unique nutritional and health values. Among these active substances, phycocyanin and polysaccharides are the focus of current research^[1]. The content of spirulina protein is as high as 50%-70% in spirulina, which is composed of 18 kinds of amino acids. Among them, the content of 8 amino acids necessary for human body and animals is close to the standard recommended by Food and Agriculture Organization of the United Nations. It is an edible protein resource with great development value in nature, which plays a certain role in medical care^[2]. PC has more significant physiological activities and various medicinal values, such as preventing and treating tumors, regulating

blood pressure, promoting animal cell regeneration, improving the immunity, etc., and PC has a broad application prospect in functional research and development. For example, Schwartz et al. found that PC has an inhibitory effect on tumor cells^[3].

The PSP is a non-toxic water-soluble natural product composed of D-mannose, D-glucose, D-galactose and glucose^[4]. Although the research on the biological activity of PSP started relatively late, in recent years, a large number of research results have shown that PSP have anti-radiation and anti-tumor effects. In addition, the PSP exhibits the effects of improving the activity of nucleic acid endonuclease, promoting repair and improving the body's immunity^[5-6].

Therefore, in this paper, the anti-tumor immune effects of PC and PSP on S180 ascites tumor mice were compre-

hensively discussed from the levels of organs, cells and molecules, so as to provide more evidence for the medical value of spirulina and its bioactive substances.

2. Materials and Methods

2.1 Materials and Equipment

Spirulina plantensis were provided by the laboratory. BALB/C pure line mice were purchased from the Animal Department of Medical College of Qingdao University. Con A, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and HRP-AB were purchased from Sigma Company. Ultraviolet spectrophotometer (Shimadzu, Japan); Automatic labeling instrument (Bio-RAD Corporation, USA); High speed cryogenic refrigerated centrifuge type 3K30 (PE Corporation of America); Model 311 CO₂ incubator (Forma Inc.).

2.2 Methods

2.2.1 Extraction of PC

PC was extracted by cyclic freezing-thawing of phosphate buffer solution (PBS), 50 g of spirulina were added in 300 mL PBS (pH 7.0, 10 mmol/L). The solution was frozen at -20°C for 30min, thawing at a constant temperature of 38°C for 2h, and then freeze-thawing for 2 h. Subsequently, the solution was allowed to stand for 30min, and then centrifuged at 4000 r/min for 20min. The supernatant was salted out with 50% saturation of ammonium sulfate, the precipitate was dissolved with 10 mL of PBS. The solution was placed in 2000 mL of PBS (pH 7.0) for 12h dialysis to remove the ammonium ions (detected by naisonite reagent). The PC concentration in the extraction solution was calculated according to the following formula: $C \text{ (g/mL)} = (1.45 \times OD_{280} - 0.74 \times OD_{260}) \times \text{dilution times}$.

2.2.2 Extraction of PSP

50 g of spirulina were add in seven times the volume of water, and the pH of solution were reached to 10 at 80 °C for 4h of water bath. Oxalic acid were added to adjust pH to 7, adding 3% TAC, and then the solution were centrifuged. The supernatant was added with 5 times volume alcohol, the precipitate was washed with acetone and freeze-dried to obtain white powdery polysaccharide. 10 g/L mother liquor were prepared with double steamed water, and stored at 4°C for standby use.

2.2.3 Anti-tumor Effect

21 BALB/C mice (weight 18-22g, half male and half female) were taken, and ascites tumor cells (3×10^6) were injected subcutaneously into axilla of each mouse to pre-

pare S180 tumor animal model. Tumor-bearing mice were randomly divided into 3 groups. In the control group, 0.5 mL PBS was injected intraperitoneally every day. In the PC group, 0.5 ml of PC (about 30 mg/mL) was intraperitoneally injected daily. In the PSP group, 0.5 mL (about 30 mg/mL) of PSP were intraperitoneally injected daily. On the 6th day, the mice were intraperitoneally injected with 0.5 mL of 2% sheep red blood cell (SRBC). On the 11th day, the mice were killed to observe the size and weight of the tumor mass. The immune factor were detected.

2.2.4 Immune Factor Detection

Immune factors and tumor necrosis factor of mice were detected by ELISA kits, and the operation was carried out in strict accordance with the kit instructions.

3. Results and Discussion

3.1 Extraction of PC and PSP

The results showed that $OD_{260} = 0.587$ and $OD_{280} = 0.510$. When the extraction volume was 18.2 mL, the concentration of PC was $C = 30.512 \text{ mg/mL}$, so the weight of the extracted PC was 0.555 g and the extraction rate was 1.11%. The purity of PSP was 93.8% and the extraction rate was 1.30%.

3.2 Anti-tumor Effect

In order to determine the effect of PC and PSP on the growth of tumor cells, tumor diameter and mass were compared between the control group and the two experimental groups. As shown in Table 1, PC had an obvious anti-tumor effect compared with the control group, and $P < 0.01$ was statistically significant. PSP also had a strong anti-tumor effect compared with the control group ($P < 0.05$). So, the anti-tumor effect of PC group was more significant than that of PSP group.

Table 1. Influence of PC and PSP on diameter and weight of tumor lump

Group	Diameter/cm (x±s)	P	Weight/g (x±s)	P
Control group	1.244 ±0.010		1.738±0.099	
PC group	0.127±0.035	<0.01	0.055±0.044	<0.01
PSP group	0.837±0.036	<0.05	1.274±0.064	<0.05

3.3 The Immune Response of PC and PSP

The thymus and spleen weights of the control group and the two groups were compared to detect the effects of PC and PSP on the growth of immune organs in tumor-bearing mice. As shown in Table 2, both PC and PSP

can promote the development of immune organs in the body, thus enhancing the immune function of the body, but PC was more effective than PSP. The effects of PC and PSP on T lymphocyte activity in the control group and two experimental groups were determined by specific rosette formation test (SRFT)^[7], lymphocyte transformation test and proliferation reaction of spleen cells and thymus cells (MTT)^[8]. As shown in Table 3, both PC and PSP can promote the transformation of T lymphocytes into T lymphocytes, which can significantly enhance the cellular immune activity of the body. Compared with the control group, the PC group ($P < 0.01$) and PSP group ($P < 0.05$) have significant differences. Subsequently, the effects of PC and PSP on activity of B cells and antibody production were determined at cellular and molecular levels by quantitative hemolytic spectrophotometry (QHS), serum lectin test and ELISA. As shown in Table 4, both PC and PSP can enhance the activity of antibody-producing cells (B cells), and the production of antibodies were significantly increase, thus enhancing the humoral immune effects of the body. Compared with the control group, $P < 0.01$, the difference was very significant in PC and PSP group.

Table 2. Influence of PC and PSP on immune organ of mice with tumor

Group	Weight of thymus (x±s)	P	Weight of spleen (x±s)	P
Control group	0.200 ± 0.010		1.126 ± 0.109	
PC group	0.529 ± 0.008	<0.01	1.821 ± 0.064	<0.01
PSP group	0.404 ± 0.011	<0.05	1.556 ± 0.041	<0.05

Table 3. Influence of PC and PSP on the activity of T cells

Group	Spleenocyte (SRFT)	Thymocyte (SRFT)	Transformation rate	Spleenocyte OD	Thymocyte OD
Control group	18537 ± 357	12801 ± 273	41.2 ± 1.9	0.978 ± 0.069	0.744 ± 0.188
PC group	22732 ± 497	22732 ± 492	52.9 ± 3.9	1.377 ± 0.130	3.038 ± 0.132
PSP group	20375 ± 398	20375 ± 384	47.4 ± 1.7	1.127 ± 0.087	1.167 ± 0.069

Table 4. Influence of PC and PSP on cells producing antibodies

Group	Spleenocyte (OD)	Thymocyte (OD)	Content of sperm agglutinin	ELISA (OD)
Control group	0.126 ± 0.002	0.380 ± 0.015	11.200 ± 4.380	0.493 ± 0.009
PC group	0.335 ± 0.012	0.536 ± 0.011	41.142 ± 15.614	0.523 ± 0.005
PSP group	0.218 ± 0.014	0.415 ± 0.008	22.400 ± 8.764	0.509 ± 0.003

Tumor necrosis factor- α (TNF- α) is an important anti-tumor cytokine that causes tumor cell necrosis by binding to TNF receptor on the tumor cell surface^[9]. As shown

in Figure 1, the content of TNF- α in the serum, spleen cells cultured and thymus cells cultured of tumor-bearing mice, which was from PC group and the PSP group, were significantly higher than that of in the control group. In addition, the function of PC in promoting secretion of TNF- α was slightly stronger than that of PSP.

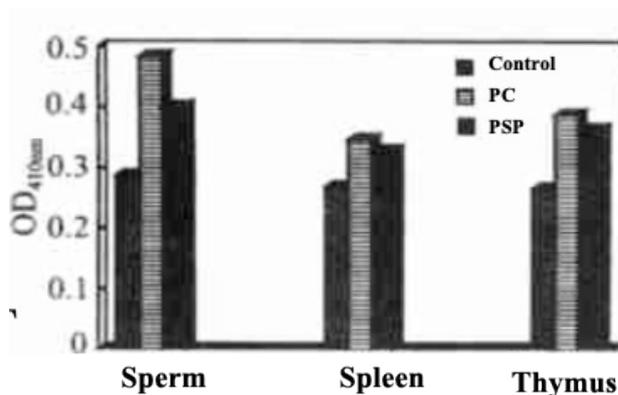


Figure 1. Determination of the content of TNF- α

4. Conclusion

In recent years, it is a new field of natural medicine research to find high effective and low toxic antitumor drugs from marine organisms. With the deepening of marine biopharmaceutical research, it has been found that many marine organisms, especially many kinds of algae. They contain a variety of unique drugs in the body function, such as antiviral, anti-tumor, anti-AIDS and bioactive components of health care on the cardiovascular and cerebrovascular system^[10]. Over the last 10 years, numerous studies have been conducted in the United States, Japan, Germany and other countries on the health functions of spirulina. And the results show that its unique protein, beta-carotene, significantly inhibits tumorigenesis, and spirulina polysaccharide also has a good effect on cancer prevention and treatment^[11]. In the study, the anti-tumor effect of PC and PSP were detected. The results showed that PC and PSP mainly achieved the function of killing tumor cells and immune regulation through the immune network induced by TNF- α with other cytokines and soluble immune molecules.

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