Effect of polysaccharide from *spirulina platensis* on hematopoietic recovery and related cytokines in mice with transplant tumor after treated by chemotherapy

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ABSTRACT **AIM**: To evaluate the effect of polysaccharide from *spirulina platensis* (PSP) on hematopoietic recovery and related cytokines in mice with transplant tumor after treated by chemotherapy. **METHODS**: The tumor cells were subcutaneously injected in the right forefoot to induce transplant solid tumor in mice. The mice were ig with PSP for 10 d and injected intraperitoneally with cyclophosphamide (CTX) for 3 d. On d 11, the count of peripheral blood cell, nucleated cell in bone marrow and CFU-S (Colony Forming Unit-Spleen) was observed; The content of DNA in bone marrow was inspected by UV-spectrophotometer; The cytokines in serums were inspected by double antibody sandwich ELISA. **RESULTS**: CTX could induce evident myelosuppression. But PSP could elevate the level of peripheral blood cell, increase nucleated cell and DNA in bone marrow, and promote CFU-S formation. In addition, PSP could also increase the content of IL-1, IL-3, GM-CSF, and TNF-α in serum. **CONCLUSION**: It is probably that PSP accelerates the hematopoietic recovery in mice with transplant tumor treated by chemotherapy by promoting endogenous cytokines secretion.

KEY WORDS polysaccharide; *spirulina platensis*; transplant tumor; myelosuppression; hematopoietic recovery; cytokines

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To date, chemotherapy is still one of major methods in tumor therapy. Bone marrow is more sensitive to chemotherapy. Besides kills tumor cells, chemotherapy also induces evident myelosuppression that is reflected in a substantial decrease and apoptosis of hematopoietic cells. The regulation of cytokines to hematopoietic system is the heat dot of research and positive and negative hematopoietic factors all participated in hematopoietic regulation[1]. Polysaccharide from *spirulina platensis* (PSP) is a natural material extract from *spirulina platensis*. It is reported that PSP has anti-aging, anti-irradiation, anti-tumor and promoting synthesis of DNA[2], and can promote hematopoietic in normal and chemotherapy injured mice in previously research[3,4]. But the machanism of PSP in promoting hematopoietic restoration after chemotherapy is still unclear, and the related research about animal bearing tumor is not reported. To further explored the mechanism, this experiment observed the effect of PSP on hematopoietic recovery and related cytokines in mice with transplant tumor after administrated with chemotherapy.

1 MATERIALS AND METHODS

1.1 Materials PSP were supplied by China Pharmaceutical University, PS acounted for 84.3% (№971622). Cyclophosphamide (CTX) was produced by Shanghai Hualian Medicine Co Ltd (№001113). ELISA Kit for mouse interleukin-1 (IL-1), IL-3, granulocyte-macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor-α (TNF-α) were purchased from Shanghai Sengxiong Biotech Industry Co Ltd.

1.2 Animal Either sex ICR mice (8-week-old, 18—20 g) were supplied by the experimental animal center of Yangzhou University (grade Ⅱ, certificate №003). The animals were allowed free access to food and water during experiment.

1.3 Tumor line Heps hepatocarcinoma cell line, provided by Shanghai Pharmacological Institute, Chinese Academy of Sciences.

1.4 Modelling and grouping 2.0×10^6 tumor cells were subcutaneously injected in the right forefoot to induce transplant solid tumor in mice. Then the mice were divided into 5 groups. In addition, normal control group was added. The control and CTX groups were ig given distilled water. The groups of large dose, moderate dose, small dose of PSP were ig given PSP 200, 100, and 50 mg·kg^{-1}·d^{-1}, respectively. Drugs were administrated at 24 h after plating tumor cells and all those drugs administration were kept on 10 d. After consecutive administration for 3 d, except for the control groups. All the mice were injected intraperitoneally CTX at the dose of 80 mg·kg^{-1}·d^{-1} for 3 d.

1.5 Peripheral blood cells count On d 11, the vein blood was obtained from orbital cavity and counted the white blood cell (WBC), the red blood cell (RBC), the hemoglobin (Hb) and the blood plates cells (BPC).

1.6 Marrow proliferation ability assay Marrow proliferation ability was indicated by DNA and nucleated cells in bone marrow. After drugs ig administrated for 10 d, the mice were sacrificed and the femurs of both sides were obtained to determine DNA in bone marrow and counted the nucleated cells in bone marrow. DNA in bone marrow determination: after got the right femur, the cavity of femur were washed with 10 ml $CaCl_2$ with a sterile syringe and a 26-gauge needle. Then placed it to 4 ℃ icebox for 30 min, centrifuged at 2 500× g for 15 min, casted off the supernatant fraction. Added 5 ml 0.2 mol·L^{-1} $HClO_4$ in the precipitant and intermixed thoroughly, heated at 90 ℃ for 15 min. The liquid were passed through stainless steel meshnets after cooling. And the optical density the liquid were measured under UV-spectrophotometer at 260 nm (1 OD260= 50 mg·L^{-1} dsDNA. DNA in per femur = OD value× 50× 5 μg). Nucleated cells in bone marrow count: the left femur was obtained and used 10 ml 3% acetic acid to wash the cavity of femur to get the bone marrow cells. Cell counts were obtained using a hemocytometer.

1.7 Hematopoietic stem cells proliferation assay Endogenous spleen colony assay was used[5]. The spleens were removed on d 11, fixed in Boun's solution, and macroscopically visible colonies would be counted and scored as colony forming unit in spleen (CFU-S).

1.8 Cytokines assay The cytokines in serums was inspected by double antibody sandwich ELISA.

1.9 Statistical analysis Data were expressed as $\bar{x}\pm s$ and analyzed by t-test. $P<0.05$ was considered as significant difference.

2 RESULTS

2.1 Effect of PSP on the decrease of peripheral blood cells induced by CTX CTX caused the decrease of peripheral blood cells including WBC, RBC, Hb and BPC. PSP could increase WBC, RBC and Hb in peripheral blood, and the effect of 200 mg·kg^{-1} was better than that of 100 mg·kg^{-1} and 50 mg·kg^{-1}. But PSP had no effect on the decrease of BPC. The effect of PSP on peripheral blood cells successively expressed as: WBC> RBC, Hb> BPC (Tab 1).

Tab 1 Effect of PSP on the decrease of peripheral blood cells induced by CTX ($\bar{x}\pm s$, n= 10)

Group	WBC /10^9·L^{-1}	RBC /10^{12}·L^{-1}	Hb /g·L^{-1}	BPC /10^9·L^{-1}
Normal control	9.8±2.9	7.9±0.8	133.7±13.4	662.4±230.4
Tumor control	9.8±2.3	7.5±0.5	126.1±7.9	659.2±164.8
CTX 80 mg·kg^{-1}× 3 d	6.5±1.8^{be}	5.9±1.0^{cf}	99.2±16.0^{cf}	392.6±191.3^{bf}
CTX+ PSP 50 mg·kg^{-1}	10.5±4.1^{h}	6.7±0.6^{dh}	113.0±10.7^{dh}	466.5±116.9^{bf}
CTX+ PSP 100 mg·kg^{-1}	11.7±4.8^{h}	7.0±0.6^{ci}	117.6±10.3^{ci}	435.7±218.2^{be}
CTX+ PSP 200 mg·kg^{-1}	13.1±2.6^{hfi}	7.4±0.6^{i}	124.5±9.4^{i}	436.3±110.8^{bf}

$^{b}P<0.05$, $^{c}P<0.01$ *vs* Normal control group; $^{e}P<0.05$, $^{f}P<0.01$ *vs* Tumor control group; $^{h}P<0.05$, $^{i}P<0.01$ *vs* CTX group

2.2 Effect of PSP on the decrease of DNA and nucleated cells in bone marrow induced by CTX CTX

significantly decreased the DNA and nucleated cells in bone marrow, and induced evident myelosuppression. PSP could increase the DNA and nucleated cells in the suppressive state. The effect of large dose group was better than that of moderate and small dose group (Tab 2).

Tab 2 Effect of PSP on the decrease of DNA and nucleated cells in bone marrow induced by CTX ($\bar{x}\pm s$, $n=8$)

Group	Nucleated cells $\times 10^6$/femur	DNA $\times$ g/femur
Normal control	12.1±2.1	376.6±33.4
Tumor control	12.4±2.3	314.4±26.6[c]
CTX 80 mg·kg$^{-1}\times$ 3d	8.3±1.2[cf]	239.3±54.6[cf]
CTX+ PSP 50 mg·kg^{-1}	9.6±1.4[be]	288.0±30.5[ch]
CTX+ PSP 100 mg·kg^{-1}	10.4±2.6	291.1±36.0[ch]
CTX+ PSP 200 mg·kg^{-1}	13.7±2.9[i]	329.1±76.1[h]

[b] $P<0.05$, [c] $P<0.01$ *vs* Normal control group; [e] $P<0.05$, [f] $P<0.01$ *vs* Tumor control group; [h] $P<0.05$, [i] $P<0.01$ *vs* CTX group

2.3 Effect of PSP on CFU-S in mice bearing Heps treated by chemotherapy CTX could significantly inhibit CFU-S formation. Whereas, three doses of PSP can all remove the inhibition induced by CTX (Tab 3).

Tab 3 Effect of PSP on CFU-S in mice bearing Heps treated by chemotherapy ($\bar{x}\pm s$, $n=10$)

Group	CFU-S(number)
Normal control	4.9±1.8
Tumor control	4.3±2.1
CTX 80 mg·kg$^{-1}\times$ 3 d	2.3±1.0[ce]
CTX+ PSP 50 mg·kg^{-1}	4.2±1.7[i]
CTX+ PSP 100 mg·kg^{-1}	4.7±1.9[i]
CTX+ PSP 200 mg·kg^{-1}	5.0±2.0[i]

[c] $P<0.01$ *vs* Normal control group; [e] $P<0.05$ *vs* Tumor control group; [i] $P<0.01$ *vs* CTX group

2.4 Effect of PSP on related cytokines in serum CTX could induce significantly decrease of IL-1 and IL-3 in serum, and there were significant difference when compared with control groups ($P<0.01$); CTX also induced slight decrease of GM-CSF and increase of TNF-α in serum, but there were no significant difference when compared with control groups ($P>0.05$). PSP could evidently elevate the content of IL-1, IL-3 and GM-CSF in serum and the effect of PSP on GM-CSF in serum was more evident. PSP 200 mg·kg^{-1} had no effect on TNF-α in serum, but there was an increase trend with the dose increase (Tab 4).

Tab 4 Effect of PSP on related cytokines in serum (ng·L^{-1}, $\bar{x}\pm s$)

Group	n	IL-1	IL-3	GM-CSF	TNF-α
Normal control	4	170.0±11.1	231.2±8.2	24.5±11.6	464.2±45.6
Tumor control	6	166.6±8.8	210.8±35.8	20.5±3.7	471.7±46.3
CTX 80 mg·kg$^{-1}\times$ 3d	6	143.2±1.3[cf]	138.7±8.9[cf]	17.4±2.8	496.3±125.6
CTX+ PSP 50 mg·kg^{-1}	6	157.4±5.8[i]	177.6±10.8[ci]	44.9±17.3[eh]	497.2±85.0
CTX+ PSP 100 mg·kg^{-1}	6	165.6±11.4[i]	184.4±11.2[ci]	44.4±15.3[eh]	496.0±104.3
CTX+ PSP 200 mg·kg^{-1}	6	171.3±12.3[i]	190.1±21.7[bi]	85.3±18.4[ci]	575.2±105.7

[b] $P<0.05$, [c] $P<0.01$ *vs* Normal control group; [e] $P<0.05$, [f] $P<0.01$ *vs* Tumor control group; [h] $P<0.05$, [i] $P<0.01$ *vs* CTX group

3 DISCUSSION

Ogawa approximately divided hematopoitic growth factors into three categories: (1) early acting factors, including stem cell factor (SCF), FLT-3 ligand (FL), IL-1, IL-11, leukemia inhibitory factor (LIF), and so on, primarily mobilized cell cycle and pomoted proliferation and differention of primitive hematopoitic stem cells; (2) intermediate acting lineage-nonspecific factors, including IL-3, GM-CSF, and so on, chiefly supported proliferation and differention of multipotential progenitors; (3) late acting lineage-specific factors, including granulocyte colony stimulating factor(G-CSF), erythropoietin(EPO), thrombopoietin(TPO), and so on, mainly supported proliferation and maturation of committed progenitors[6]. Formerly, a number of inhibitory factors had been proposed as physiologic regulators of hematopoietic and appear to be effective at very low concentrations. They include tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interferon (IFN), macrophage inflammatory protein-1α (MIP-1α), and so on. Lately, a mount of research have confirmed that these factors maintained the

stem cells in G_0 mainly by blocking the entrance of hematopoietic stem cells into the cell cycle so that protected hematopoietic stem cells from the cytotoxicity of chemotherapeutic agents and lightened the myelosuppression[1,6,7].

This experiment confirmed that CTX can induce evident myelosuppression and PSP can accelerate the hematopoietic recovery. In addition, CTX can induce significant decrease of IL-1 and IL-3 and slight decrease of GM-CSF and increase of TNF-α in serum. The reason could be that chemotherapy mobilized primitive hematopoitic stem cells and progenitor cells, which inhibited early and intermediated acting factors secretion in a negative feedback manner, and promoted negative growth factors secretion to antagonize the mobilization of primitive hematopoitic stem cells and progenitor cells induced by chemotherapy. PSP can evidently elevate the content of IL-1, IL-3 and GM-CSF in serum. However, PSP has no effect on TNF-α in serum, but there is an increase trend with an increase in the doses of PSP. These results indicate that PSP promotes endogenous positive and negtive cytokines secretion and the effect on positive cytokines are more evident. This consists with Grzegorzewski' hypothesis that jiontly using positive and negtive cytokines can achieve more effective chemoprotection. Namely, positive regulators of hematopoietic, such as IL-1, IL-6, CSFs and/or SCF could be used to stimulate the proliferation and/or production of critical stem, progenitor, or end-stage hematopoietic cells. This approach could result in a large pool of these cells before chemotherapy, thus allowing a more rapid recovering. Alternatively, they may be used posttreatment to restimulate the remains of the stem/progenitor compartment after myelosuppression. Alternatively the number of critical stem/progenitor cells could be increased and then a potent negative regulator of hematopoietic used to temporarily inhibit their proliferation thereby rendering them more resistant to killing by chemotherapeatic drugs[11]. However, in Grzegorzewski' hypothesis, it is difficult to master the time and dosage of the negtive growth factors be administrated, if we use exogenous cytokines to regulate hematoposis. Ogawa ever supposed that negative growth factors appear to be effective at very low concentrations[6]. Recently, Batard reported that TGF β_1 at low physiologic concentration especially maintained human primitive hematopoitic stem cells in G_0 and did not induce apotosis[8]. All these experimental results suggest PSP promotes endogenous positive and negtive cytokines secretion and this intrinsic alliance can more completely regulate hematopoietic recovery. Moreover, IL-1 and TNF-α also induce bone marrow cells to express mRNA for MnSOD and overexpression of MnSOD can increase resistance to chemotherapy. In addition, IL-1 increase the synthesis of NADPH of BM cells, However MnSOD and NADPH are important to scavenge free radical[9].

In conclusion, it is probably that PSP alleviates myelosuppression and accelerates the hematopoietic recovery in mice with transplant tumor administrated with chemotherapy by promoting endogenous cytokines secretion. As for the mechanisms of cytokines implementing the chemoprotection, it is necessary to further research.

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螺旋藻多糖对小鼠移植性肿瘤化疗后造血恢复及相关细胞因子的影响

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摘要 目的: 研究螺旋藻多糖(polysaccharide from *spirulina platensis*, PSP)对小鼠移植性肿瘤化疗后造血恢复及相关细胞因子的影响。方法: 通过右前肢皮下接种肿瘤细胞形成小鼠移植性实体瘤, 给予PSP连续灌胃 10 d, d 4 给予环磷酰胺(CTX)腹腔注射连续 3 d。于 d 11, 分别观察了外周血细胞、骨髓有核细胞及CFU-S计数, 用紫外分光光度计检测骨髓DNA含量, 用双抗体夹心ELISA法检测血清中IL-1、IL-3、GM-CSF、TNF含量。结果: CTX可造成明显骨髓抑制, 而PSP则提升了外周血细胞、增加了骨髓中有核细胞计数和DNA含量以及促进了CFU-S形成。此外, PSP还增加了血清中IL-1、IL-3、GM-CSF、TNFα含量。结论: PSP可能通过促进内源性细胞因子的分泌来实现其促进小鼠移植性肿瘤化疗损伤后的造血恢复。

关键词 螺旋藻多糖; 移植性肿瘤; 骨髓抑制; 造血恢复; 细胞因子

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